*Available online a*t www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2009, 1 (2) 143-150
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071

Transferosomes: A Novel Approach for Transdermal Drug Delivery

Pandey Shivanand*, Goyani Manish, Devmurari Viral, Fakir Jarina

Smt. R. B. Patel Mahila Pharmacy, Atkot, Rajkot, Gujarat, India

Abstract

Poor patient compliance is a frequent problem in daily clinical practice. The unfavorable pharmacokinetic of the drug, the inconveniences of the standard from of such drug application and the side effects due to the administration route often are the reasons for this. The high and self-optimizing deformability of typical composite transfersomes membrane, which are adaptable to ambient tress allow the ultra deformable transfersomes to change its membrane composition locally and reversibly, when it is pressed against or attracted into narrow pore. The transfersomes components that sustain strong membrane deformation preferentially accumulate, while the less adaptable molecules are diluted at sites of great stress. This dramatically lowers the energetic cost of membrane deformation and permits the resulting, highly flexible particles, first to enter and then to pass through the pores rapidly and efficiently. This behavior is not limited to one type of pore and has been observed in natural barriers such as in intact skin. Transfersomes possess an infrastructure consisting of hydrophobic and hydrophilic moieties together and as a result can accommodate drug molecules with wide range of solubility. Transfersomes can deform and pass through narrow constriction (from 5 to 10 times less than their own diameter) without measurable loss. This high deformability gives better penetration of intact vesicles. They can act as a carrier for low as well as high molecular weight drugs e.g. analgesic, anesthetic, corticosteroids, sex hormone, anticancer, insulin, gap junction protein, and albumin.

Keywords: transfersomes, pharmacokinetic, analgesic, anesthetic, corticosteroids

Introduction

Delivery via the transdermal route is an interesting option in this respect because a transdermal route is convenient and safe. This offers several potential advantages over conventional routes [1] like avoidance of first pass metabolism, predictable and extended duration of activity, minimizing undesirable side effects, utility of short half-life drugs, improving physiological and pharmacological response, avoiding the fluctuation in drug levels, inter-and intra-patient variations, and most importantly, it provides patients convenience. To date many chemical and physical approaches have been applied to increase the efficacy of the material transfer across the intact skin, by use of the penetration enhancers, enhancers, iontophoresis, sonophoresis and the use of colloidal carriers such as lipid vesicles (liposomes and proliposomes) and nonionic surfactant vesicles (niosomes and proniosomes).

Transfersomes were developed in order to take the advantage of phospholipids vesicles as transdermal drug carrier. These self-optimized aggregates, with the ultra flexible membrane, are able to deliver the drug reproducibly either into or through the skin, depending on the choice of administration or application, with high efficiency. These vesicular transfersomes are several orders of magnitudes more elastic than the standard liposomes and thus well suited for the skin penetration. Transfersomes overcome the skin penetration difficulty by squeezing themselves along the intracellular sealing lipid of the stratum corneum. There is provision for this, because of the high vesicle deformability, which permits the entry due to the mechanical stress of surrounding, in a self-adapting manner. Flexibility of transfersomes membrane is achieved by mixing suitable surface-active components in the proper ratios [2]. The resulting flexibility of transfersome membrane minimizes the risk of complete vesicle rupture in the skin and allows transfersomes to follow the natural water gradient across the epidermis, when applied under nonocclusive condition. Transfersomes can penetrate the intact stratum corneum spontaneously along two routes in the intracellular lipid that differ in their bilayers properties [3]. The following figure shows possible micro routes for drug penetration across human skin intracellular and transcellular [4].

The high and self-optimizing deformability of typical composite transfersomes membrane, which are adaptable to ambient tress allow the ultra deformable transfersomes to change its membrane composition locally and reversibly, when it is pressed against or attracted into narrow pore. The transfersomes components that sustain strong membrane deformation preferentially accumulate, while the less adaptable molecules are diluted at sites of great stress. This dramatically lowers the energetic cost of membrane deformation and permits the resulting, highly flexible particles, first to enter and then to pass through the pores rapidly and efficiently. This behavior is not limited to one type of pore and has been observed in natural barriers such as in intact skin [5, 6].

***Salient Features and Limitations of Transresomes*:** Transfersomes possess an infrastructure consisting of hydrophobic and hydrophilic moieties together and as a result can accommodate drug molecules with wide range of solubility. Transfersomes can deform and pass through narrow constriction (from 5 to 10 times less than their own diameter) without measurable loss. This high deformability gives better penetration of intact vesicles. They can act as a carrier for low as well as high molecular weight drugs e.g. analgesic, anesthetic, corticosteroids, sex hormone, anticancer, insulin, gap junction protein, and albumin. They are biocompatible and biodegradable as they are made from natural phospholipids similar to liposomes. They have high entrapment efficiency, in case of lipophilic drug near to 90%. They protect the encapsulated drug from metabolic degradation. They act as depot, releasing their contents slowly and gradually. They can be used for both systemic as well as topical delivery of drug. Easy to scale up, as procedure is simple, do not involve lengthy procedure and unnecessary use or pharmaceutically unacceptable additives.

***Limitations of Transfersomes*:** Transfersomes are chemically unstable because of their predisposition to oxidative degradation. Purity of natural phospholipids is another criteria militating against adoption of transfersomes as drug delivery vehicles. Transfersomes formulations are expensive.

***Transfersomes Vs Other Carrier Systems*:** At first glance, transfersomes appear to be remotely related to lipid bilayers vesicle, liposomes. However in functional terms, transfersomes differ vastly from commonly used liposomes in that they are much more flexible and adaptable. The extremely high flexibility of their membrane permits transfersomes to squeeze themselves even through pores much smaller than their own diameter. This is due to high flexibility of the transfersomes membrane and is achieved by judiciously combining at least two lipophilic/amphiphilic components (phospholipids plus biosurfactant) with sufficiently different packing characteristics into a single bilayer. The high resulting aggregate deformability permits transfersomes to penetrate the skin spontaneously. This tendency is supported by the high transfersomes surface hydrophilicity that enforce the search for surrounding of high water activity. It is almost certain that the high penetration potential of the transfersomes is not primarily a consequence of stratum corneum fluidization by the surfactant because micellar suspension contains much more surfactant than transfersomes (PC/Sodium cholate 65/35 w/w %, respectively). Thus, if the penetration enhancement via the solubilization of the skin lipids was the reason for the superior penetration capability of transfersomes, one would expect an even better penetration performance of the micelles. In contrast to this postulate, the higher surfactant concentration in the mixed micelles does not improve the efficacy of material transport into the skin. On the contrary, mixed micelles stay confined to the topmost part of the stratum corneum even they are applied non occlusively [7]. The reason for this is that mixed micelles are much less sensitive to the transepidermal water activity gradient than transfersomes. Transfersomes differ in at least two basic features from the mixed micelles, first a transfersomes is normally by one to two orders of magnitude (in size) greater than standard lipid micelles. Secondly and more importantly, each vesicular transfersomes contains a water filled core whereas a micelle is just a simple fatty droplet. Transfersomes thus carry water as well as fat-soluble agent in comparison to micelles that can only incorporate lipoidal substances [8, 9].

To differentiate the penetration ability of all these carrier systems [10] proposed the distribution profiles of fluorescently labeled mixed lipid micelles, liposomes and transfersomes as measured by the Confocal Scanning Laser Microscopy (CSLM) in the intact murine skin. In all these vesicles the highly deformable transfersomes transverse the stratum corneum and enter into the viable epidermis in significant quantity.

Chapman & Walsh [11] also showed that the former two types of aggregates are confined to the outer half of the horny layer, where the cellular packing and intercellular seals are already compromised by the desquamation process. Pure lipid vesicles or micelles seem to have access to the low-resistance pathway only and thus very seldom reach the lower stratum corneum or even get into the viable part of the skin in significant quantities.

Method	Advantage	Disadvantage
Penetration enhancers (Walters, 1989)	Increase penetration through skin and give both local and systemic effect	Skin irritation Immunogenicity, only for low molecular weight drugs
Physical methods e.g. Iontophoresis (Cevc et al, 1995	Increase penetration of intermediate size charged molecule	Only for charged drugs, transfer efficiency is low (less than 10%)
Liposomes (Hadgraft & Guy, 1989)	Phospholipid vesicle, biocompatible, biodegradable	Less skin penetration less stable
Proliposome	Phospholipid vesicle, more stable	Less penetration, cause aggregation

	than liposomes	and fusion of yesicles
Niosomes (Schreier & Bouwstra, 1994) (Holland et al, 1995) Proniosomes	Non-ionic surfactants vesicles, greater stability, Will convert into niosome in situ, stable	Less skin penetration easy handling But will not reach upto deeper skin layer
Transfersomes and Protransfersomes (Cevc et al, 1996)	More stable, high penetration due to high deformability, biocompatible and biodegradable, suitable for both low and high molecular weight and also for lipophilic as well as hydrophilic drugs and reach upto deeper skin layers.	None, but for some limitations

***Mechanism of Penetration of Transfersomes*:** Transfersomes when applied under suitable condition can transfer 0.1 mg of lipid per hour and cm2 area across the intact skin. This value is substantially higher than that which is typically driven by the transdermal concentration gradients. The reason for this high flux rate is naturally occurring "transdermal osmotic gradients" i.e. another much more prominent gradient is available across the skin [2]. 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 (75% water content) and nearly completely dry stratum corneum, near to the skin surface (15% water content) [3]. This gradient is very stable because ambient air is a perfect sink for the water molecule even when the transdermal water loss is unphysiologically high. All polar lipids attract some water this is due to the energetically favourable interaction between the hydrophilic lipid residues and their proximal water. Most lipid bilayers thus spontaneously resist an induced dehydration [4, 5]. Consequently all lipid vesicles made from the polar lipid vesicles move from the rather dry location to the sites with a sufficiently high water concentration [6, 7] So when lipid suspension (transfersomes) is placed on the skin surface, that is partly dehydrated by the water evaporation loss and then the lipid vesicles feel this "osmotic gradient" and try to escape complete drying by moving along this gradient [3]. They can only achieve this if they are sufficiently deformable to pass through the narrow pores in the skin, because transfersomes composed of surfactant have more suitable rheologic and hydration properties than that responsible for their greater deformability [1, 7] less deformable vesicles including standard liposomes are confined to the skin surface, where they dehydrate completely and fuse, so they have less penetration power than transfersomes. Transfersomes are optimized in this respect and thus attain maximum flexibility, so they can take full advantages of the transepidermal osmotic gradient (water concentration gradient) [8].

***Propensity of penetration*:** The magnitude of the transport driving force, of course, also plays an important role:

$$\text{Flow} = \text{Area} \times \text{(Barrier) Permeability} \times \text{(Trans-barrier) force.}$$

Therefore, the chemically driven lipid flow across the skin always decreases dramatically when lipid solution is replaced by the some amount of lipids in a suspension.

Materials and Methods

Materials commonly used for the preparation of transfersomes are summarized in Table

Different additives used in formulation of transfersomes [9-14]

Class	**Example**	**Uses**	**References**
Phospholipids	Soya phosphatidyl choline Dipalmitoyl phosphatidyl choline Distearoyl phoshatidyl choline	Vesicles forming component	Cevc et al, 1997 Cevc, 1992_b
Surfactant	Sod. cholate Sod.deoxycholate tween-80 Span-80	For providing flexibility	Schubert et al, 1986 Schubert et al,1988 Cevc et al, 1995 Gamal et al,199
Alcohol	Ethanol	As a solvent	Planas et al, 1992 Gamal et al,1999
Dye	Rhodamine-123 Rhodamine-DHPE Fluorescein-DHPE Nile-red	For CSLM study	Cevc et al, 1995 Schatzlein & Cevc, 1998
Buffering agent	Saline phosphate buffer (pH 6.4)	As a hydrating medium	Cevc, 1993

All the methods of preparation of transfersomes are comprised of two steps. First, a thin film is prepared hydrated and then brought to the desired size by sonication; and secondly, sonicated vesicles are homogenized by extrusion through a polycarbonate membrane. The mixture of vesicles forming ingredients, that is phospholipids and surfactant were dissolved in volatile organic solvent (chloroform-methanol), organic solvent evaporated above the lipid transition temperature (room temp. for pure PC vesicles, or 50^0C for dipalmitoyl phosphatidyl choline) using rotary evaporator. Final traces of solvent were removed under vacuum for overnight. The deposited lipid films were hydrated with buffer (pH 6.5) by rotation at 60 rpm min $^{-1}$ fir 1 hr at the corresponding temperature. The resulting vesicles were swollen for 2 hr at room temperature. To prepare small vesicles, resulting LMVs were sonicated at room temperature or 50^0C for 30 min. using a B-12 FTZ bath sonicator or probe sonicated at 4^0C for 30 min (titanium micro tip, Heat Systems W 380). The sonicated vesicles were homogenized by manual extrusion 10 times through a sandwich of 200 and 100 nm polycarbonate membrane [15].

***Characterization of Transfersomes*:** The characterization of transfersomes is generally similar to liposomes, niosomes and micelles.

***Entrapment Efficiency*:** The entrapment efficiency is expressed as the percentage entrapment of the drug added. Entrapment efficiency was determined by first separation of the unentrapped drug by use of mini-column centrifugation method . After centrifugation, the vesicles were disrupted using 0.1% Triton X-100 or 50% n-propanol. The entrapment efficiency is expressed as:

$$\frac{\text{Amount entrapped}}{\text{Total amount added}} \times 100$$

***Vesicle Diameter*:** Vesicle diameter can be determined using photon correlation spectroscopy or dynamic light scattering (DLS) method. Samples were prepared in distilled water, filtered through a 0.2 mm membrane filter and diluted with filtered saline and than size measurement done by using photon correlation spectroscopy or dynamic light scattering (DLS) measurements (Gamal et al, 1999) [16].

***Confocal Scanning Laser Microscopy (CSLM) study*:** Conventional light microscopy and electron microscopy both face problem of fixation, sectioning and staining of the skin samples. Often the structures to be examined are actually incompatible with the corresponding processing techniques; these give rise to misinterpretation, but can be minimized by Confocal Scanning Laser Microscopy (CSLM). In this technique lipophilic fluorescence markers are incorporated into the transfersomes and the light emitted by these markers used for following purpose [17]:

- for investigating the mechanism of penetration of transfersomes across the skin,
- for determining histological organization of the skin (epidermal columns, interdigitation), shapes and architecture of the skin penetration pathways. for comparison and differentiation of the mechanism of penetration of transfersomes with liposomes, niosomes and micelles.

Different fluorescence markers used in CSLM study are
I. Fluorescein-DHPE(1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine-N-(5-fluoresdenthiocarbamoyl), triethylammonium salt)
II. Rhodamine-DHPE (1,2-dihexadecanoyl-sn-glycero-3ogisogietgabikanube-N-Lissamine^{Tm}rhodamine B sulfonyl), triethanolamine salt)
III. NBD-PE (1, 2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine-N-(7-nitro-Benz-2-oxa-1, 3-diazol-4-yl) triethanolamine salt)
IV. Nile red.

***Degree of Deformability or Permeability Measurement*:** In the case of transfersomes, the permeability study is one of the important and unique parameter for characterization. The deformability study is done against the pure water as standard. Transfersomes preparation is passed through a large number of pores of known size (through a sandwich of different microporous filters, with pore diameter between 50 nm and 400 nm, depending on the starting transfersomes suspension). Particle size and size distributions are noted after each pass by dynamic light scattering (DLS) measurements.

In vitro Drug Release: In vitro drug release study is performed for determining the permeation rate. Time needed to attain steady state permeation and the permeation flux at steady state and the information from in-vitro studies are used to optimize the formulation before more expensive in vivo studies are performed. For determining drug release, transfersomes suspension is incubated at 32^{0}C and samples are taken at different times and the free drug is separated by minicolumn centrifugation (Fry et al., 1978) [18]. 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).

***In Vivo Fate of Transfersomes and Kinetics of Transfersomes* Penetration:** After having penetrated through the outermost skin layers, transfersomes reach the deeper skin layer, the dermis. From this latter skin region they are normally washed out, via the lymph, into the blood circulation and through the latter throughout the body, if applied under suitable conditions. Transfersomes can thus reach all such body tissues that are accessible to the subcutaneously injected liposomes. The kinetics of action of an epicutaneously-applied agent depends on the velocity of carrier penetration as well as on the speed of drug (re)distribution and the action after this passage. The most important single factors in this process are: **a)**. Carrier in-flow. **B).** Carrier accumulation at the targets site. **C)**. Carrier elimination.

The onset of penetration-driving force depends on the volume of the suspension medium that must evaporate from the skin surface before the sufficiently strong trans-cutaneous chemical potential chemical potential or water activity gradient is established. Using less solvent is favorable in this respect. The rate of carrier passage across the skin is chiefly determined by the activation energy for the carrier deformation. The magnitude of the penetration driving force also plays a big role. This explains, for example, why the occlusion of an application site or the use of too strongly diluted suspension hampers the penetration process.

Carrier elimination from the sub cutis is primarily affected by the lymphatic flow, general anesthesia or any other factor that affects this flow, consequently, is prone to modify the rate of transcutaneous carrier transport. While it has been estimated that approximately 10% of the cardiac blood flow pass through each gram of living skin tissue, no comparable quotation is available for the lymph. Further, drug distribution is also sensitive to the number of carrier used, as this may affect the rate of vehicle degradation and / or filtration in the lymph nodes. The lag between the time of application and the time of drug appearance in the body, therefore, is always quite long, complex and strongly sensitive to the type of drug and formulation administration.

In the best case, the skin penetration lag amounts to approximately 15 min. if rapidly exchanging agents such as local analgesics are detected right under the skin permeability barrier (Planas et al., 1992) [19]. Less rapidly exchanging molecules or molecules measured in the blood compartment are typically detected with a lag time between 2 and 6 hr. depending on the details of drug formulation. Molecules that do not diffuse readily from the carriers or agents delivered with the suboptimal carriers normally fall in this category. The kinetics of vesicle penetration into and across the skin can be controlled to a large extent by fixing the physicochemical characteristics of the drug carrier suspension.

Kinetics of the transfersomes penetration through the intact skin is best studied in the direct biological assays in which vesicle associated drugs exert their action directly under the skin surface. Local analgesics are useful for this purpose, For determining the kinetics of penetration, various lidocaine loaded vesicles were left to dry out on the intact skin. Corresponding subcutaneous injection is used as control. The animal's sensitivity to pain at the treated site after each application was then measured as a function of time. Dermally applied standard drug carrying liposomes or simple lidocaine solution have never caused any analgesic effect. It was necessary to inject such agent preparations to achieve significant pain suppression. In contrast to this, the lidocaine-loaded transfersomes were analgesic ally active even when applied dermally. Maximum analgesic effect with the latter type of drug application was typically observed 15 minutes after the drug application. A marked analgesic effect was still noticeable after very long time. The precise reach as well as kinetics of transfersomes penetration through the skin are affected by: drug carrier interaction, application condition or form, skin characteristics, applied dose.

Conclusion

Transfersomes are specially optimized particles or vesicles, which can respond to an external stress by rapid and energetically inexpensive, shape transformations. Such highly deformable particles can thus be used to bring drugs across the biological permeability barriers, such as skin. When tested in artificial systems. Transfersomes can pass through even tiny pores (100 mm) nearly as efficiently as water, which is 1500 times smaller. Drug laden transfersomes can carry unprecedented amount of drug per unit time across the skin (up to 100mg cm^2h^{-1}). The systemic drug availability thus mediated is frequently higher than, or at least approaches 80-90%. The bio-distribution of radioactively labeled phospholipids applied in the form of transfersomes after 24 hr is essentially the same after an epicutaneous application or subcutaneous injection of the preparations. When used under different application conditions, transfersomes can also positioned nearly exclusively and essentially quantitatively into the viable skin region.

References

[1] Shaw,J.E; Chandrasekaran,S.K. In: pharmacology of the skin, Greaves, M.W.; Shuster S. (Eds.) Springer- Verlag, Berlin,**1999**, 115-122
[2] Cevc, G., *Chem. Phys. Lipids*, **1991,** 57:293-299
[3] Schatzlein,A; Cevc,G. Skin penetration by phospholipids vesicles, Transfersomes as visualized by means of the Confocal Scanning Laser Microscopy, In; characterization, metabolisim, and novel biological applications. Champaign. AOCS Press, **1995**, 191-209
[4] Panchagnula,R., *Ind J.Pharmacol*, **1997,** 29:140-156
[5] In: Bain, K.R.; Hadgkraft, A.J.; James, W.J.; Water, K.A.(Eds), Prediction of percutaneous penetration, **1993**, Vol. 3b, STS Publishing, Cardiff, 226-234
[6] Cevc.G; Blume.G; Sehatzlein,A.; Gebauer,D;Paul,A., *Adv.Drug. Deliv.Rev*. **1996**,18:349-378
[7] Chapman,S.J; Walsh,A., *Arch. Dermatol*, **1998,** Res. 282: 304-320
[8] Gompper. G; Kroll, D.M., *Phys. Rev*,**1995**, E.52: 4198-4211
[9] Wearner. R.R; Myers; M.C; Taylar; D.A. , *J.Inves.Dermatol.* **1988**, 90:218-224
[10] Rand,R.P.;Parsegian,V.A. , *Biochem. Biophys Acta*.**1989,** 988: 351-377
[11] Cevc. G; Blume. G.: Schatzlein, A., *J. Control. Rel*, **1997**, 45:211-226
[12] Cevc,G. Grbauer,D; Schatzlein,A; Blume,G., *Biochem. Acta*,**1998**, 1368:201-215
[13] Gamal, M; EI Maghraby, M.; Williams, A.C.; Barry B.W, *J.Pharm.Pharmacol.* **1999**, 51:1123-1134
[14] Schatzlein,A.;Cevc,G., *Brit. J. Dermatol*, **1998**, 138:583-598
[15] Fry, D.W.; White, J.C.; Goldman, I.D., *J.Anal.Biochem*, **1978**, 90:809-815
[16] Planas, M.E; Gonzalez.P; Rodriguez, S; Sanchez, G.; Cevc.G. *Anesth. Analg*. **1992**, 615-621