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A Review on Novel Vesicular Drug Delivery System: Transfersomes

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Abstract

In novel drug delivery systems, transdermal route is considered most safe and effective due to several advantages such as predictable and extended duration of action, avoidance of first pass metabolism, lesser side effects etc. However it has also got certain limitations like incompetence to overcome barriers of stratum corneum, unable to transport larger molecules and many more. To overcome all these problems, transfersomes came into existence as they possess the quality of other transdermal drug delivery systems but at the same time can also cross the barrier with ease. These transfersomes being an ultradeformable vesicle and elastic in nature can squeeze itself through a pore which is many times smaller than its size owing to its elasticity.

Hence among various vesicular systems, transfersomes have gained enormous importance in the last decade for sustained and targeted drug delivery. Knowing the potentiality of this novel vesicular drug delivery system, main focus has been given in this article towards reviewing all key aspects of a transfersomes through various literatures, along with its applications for delivery of various substances such as herbal drugs, proteins, NSAID's etc.

Keywords: Novel Drug Delivery Systems, Stratum Corneum, Transfersomes, Ultradeformable Vesicle.

Introduction

In today's scenario, research focus has been shifted from conventional to novel drug delivery systems (NDDS) so as to achieve high therapeutic activity along with better patient compliance. So far many drug delivery systems with enhanced therapeutic activity have been prepared and coming in markets such as transdermal patches, microsomes etc. This has proved the effectiveness of NDDS to develop the formulations of preexisting as well as newly found potent lead compound.[1] Over the past few years, among several novel drug delivery systems considerable attention has been given on developing the transdermal drug delivery because it overcome several problems associated with oral drug delivery system and offer number of benefits. As the skin in an average adult body covers an area of approximately 1.8m^2 and a total weight of 3 kg; it receives about one-third of the blood circulating in the body. Hence the administration of the drug for a localized effect and transdermal drug delivery system (TDDS) utilizes skin as a potential route for systemic action of the drug. But some of the major disadvantages such as the possibility of local irritation, erythema, itching and low permeability in the stratum corneum have limited their use. Moreover a serious obstacle to the drugs administration by the dermal and transdermal route are the permeation characteristics of drug substance through the stratum corneum, which limits the transport of drugs, making the route of insufficient for medical use.[2]

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Among the various TDDS, vesicular systems have gained importance over the period of time as a means for sustained and efficient drug delivery as they enhance the bioavailability of drugs especially poorly aqueous soluble drugs with reduction in the cost of therapy. Vesicular drug delivery systems such as lipososmes, niosomes, transfersomes, offer several advantages such as encapsulation of hydrophilic and lipophilic drugs, reduction in dose related adverse effects, high therapeutic efficacy of drugs for longer time period and increased time of drug presence in the circulation due to encapsulation in the vesicular structure. The most important role performed by vesicular system is to control the degradation of drug and its loss and sufficient availability of drug to the diseased site of action.[3]

In between so many vesicular systems, the elastic or deformable vesicles are a novel type of liquid-state vesicles, one of the examples of which is Transfersomes that provide a great scope for the delivery of drug substances. In year 1991, concept of transfersomes was launched by Gregor Cevc. Transferosome is a registered trademark technology of the German company IDEA AG. The name implies a "bearing body" according to the Latin and Greek names "transferred" and "soma" respectively. Transfer literally means to carry while "soma" means a body.[4,5]

Table 1: Pros and Cons of Several Vesicular Systems [6]

Methods	Advantages	Disadvantages
Liposomes	Phospholipid vesicle, biocompatible, biodegradable	Less skin penetration, less stable
Proliposome	Phospholipid vesicle, more stable than liposome	Less penetration, cause aggregation and fusion of vesicles
Niosomes	Non-ionic surfactant vesicles	Less skin penetration easy handling
Proniosommes	Convert to stable niosome in situ	Cannot reach in the deeper layers of the skin
Transfersomes and Protransfersomes	High deforming ability which ensures deeper penetration in skin layers	Very few disadvantages
Colloidosomes	Can withstand high mechanical load	Insufficient locking of drug can lead to coalescence
Cubosomes	Targeted and controlled release of materials in a biodegradable manner	No disadvantage as such is reported

In a broad sense, the transferosome is an extremely adaptable, stress-sensitive, elastic aggregate. It exists as an ultra-deformable complex that has an aqueous core surrounded by a complex layer of lipids. Its preferred form is an oversized vesicle which has an aqueous core surrounded by the lipid bilayer complex. Vesicles are water-filled colloidal particles. The walls of these capsules consist of amphiphilic molecules (lipids and surfactants) in a bilayer conformation. These vesicles serve as a depot for the sustained release of active compounds in the case of topical formulations, as well as rate-limiting membrane barrier for the modulation of systemic absorption in the case of transdermal formulations. This carrier system is composed of phospholipids, surfactants and water Transfersomes consist of a phospholipids component along with a surfactant mixture. The ratio of individual surfactants and total amount of surfactants control the flexibility of the vesicle.[7]

The interdependence between the local composition and the shape of the bilayer layer makes the vesicles self-regulating and self-optimizing. This allows transfersome to effectively overcome multiple transport barriers and thus act as a drug carrier for targeted delivery of non-invasive drugs and prolonged release of therapeutic agents.[5]

Salient Features of Transfersomes [8,9]**:**

1. Transfersomes possess an infrastructure consisting of lipophillic and hydrophilic moieties together and consequently can accommodate drug molecules with wide range of solubility.
2. Being ultrafoldable vesicles transfersomes can deform and pass through narrow constriction that is 5 to 10 times less than their own diameter without measurable loss. This high deformability provides better penetration of intact vesicles.
3. They can act as a carrier for low as well as high molecular weight drugs. For example analgesic, anaesthetic, corticosteroids, sex

hormone, anticancer, insulin, gap junction protein, and albumin.

4. They are biocompatible and biodegradable since they are made with natural phospholipids similar to liposomes.
5. In case of lipophilic drug, transfersomes shows high entrapment efficiency, nearly about 90%.
6. They protect the encapsulated drug from metabolic degradation.
7. They act as reservoir or depot, releasing their contents slowly and gradually.
8. They can be used for both systemic as well as topical delivery of drug.
9. As procedure is simple, do not involve lengthy procedure and unnecessary requirement of pharmaceutically unacceptable additives, it is easy to scale up the production process.

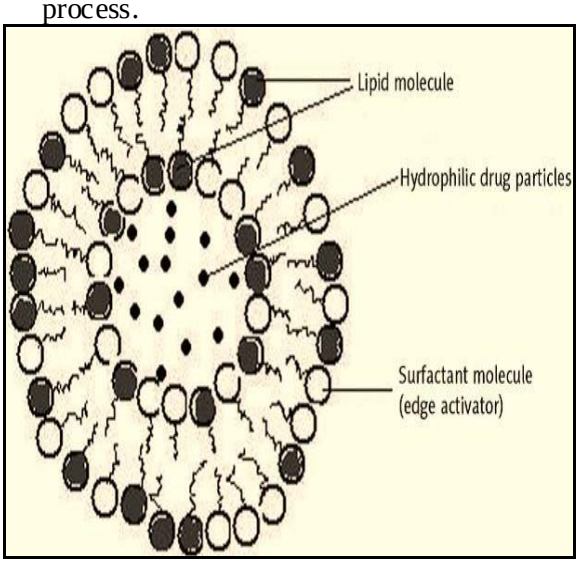

Fig. 1: Structural Representation of Transfersome Unit [7]

Advantages [10,11]**:**

1. Due to self optimized and ultra-flexible membrane properties, successively delivers drug either into or through the skin, with high efficiency.
2. An equivalent therapeutic effect can be achieved with a lower daily dose of the drug than necessary.
3. Suitable for the skin penetration and permits the entry due to the mechanical stress of surrounding, in a self-assembling manner.
4. Flexibility in membrane of transfersomes, reduces the risk of vesicle rupture in the skin and permits them to follow the natural water gradient across the epidermis when applied under non-occlusive condition.
5. Drug release rate and the deposition to the target site can be adjusted by modification of vesicular composition or surface property of transfersome membrane.
6. Provide a constant or steady infusion of drugs over a long period of time and hence maintains adequate plasma concentration of potent drugs.
7. As they provide longer duration of the action hence permits consequent reduction of the dosing frequency, thereby increasing the patient compliance.
8. Improved bioavailability and greater convenience in the administration of drugs can be acquired with transfersomes.
9. Reduced side effects and improved therapy because of plasma level maintenance, until the end of the dosage interval.
10. Avoid inter-patient and intra-patient variations and improve therapeutic efficacy.

Disadvantages [1,12]**:**

1. High cost of the product is a major drawback for the wide acceptance of this transfersomes.
2. Many drugs especially hydrophilic permeate the skin too slowly to be of therapeutic benefit.
3. Skin barrier function changes from one site to another on the same person, from person to person and also with age that causes variability in drug release.
4. Not suitable for high drug doses.
5. Hypersensitivity reactions and irritation to skin may occur.
6. Drugs that require high blood levels cannot be administered.
7. Highly susceptible to oxidative degradation that makes transfersomes unstable.
8. Purity of natural phospholipids is another criterion that poses problems in using transfersomes as drug delivery vehicles.

Scope of Transfersomes [13]**:**

Transfersome technology is best suited for non-invasive delivery of therapeutic molecules across open biological barriers. The Transfersome vesicles can transport across the skin, for example, molecules that are too big to diffuse through the barrier. Examples include systemic delivery of therapeutically meaningful amounts of

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macromolecules, such as insulin or interferon, across intact mammalian skin. Other applications include the transport of small molecule drugs which have certain physicochemical properties which would otherwise prevent them from diffusing across the barrier.

Another attraction of the Transfersome technology is the carrier's ability to target peripheral, subcutaneous tissue. This ability relies on minimisation of the carrier-associated drug clearance through cutaneous blood vessels plexus: the non-fenestrated blood capillary walls in the skin together with the tight junctions between endothelial cells preclude vesicles getting directly into blood, thus maximizing local drug retention and propensity to reach the peripheral tissue targets.

Mechanism of Transfersomes Penetration into the Skin [6,14,15]**:**

Applied under suitable conditions, transfersomes, can transfer 0.1 mg of lipid per hour and square centimeter area across the intact skin. The mentioned value is substantially higher than that typically determined by transdermal concentration gradients. Reason for this high flux rate is naturally occurring "transdermal osmotic gradients". This osmotic gradient that develops 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 the surface of the skin (water content of 15%).

This gradient is very stable because the ambient air is a perfect sink for the water molecule even when the transdermal water loss is physiologically high. All polar lipids attract some water, due to the energetically favorable interaction between the hydrophilic lipid residues and their proximal water. Therefore, most lipid bilayers spontaneously resist induced dehydration. Consequently, all lipid vesicles produced by polar lipid vesicles move from the rather dry position to sites with a sufficiently high concentration of water. So when the lipid suspension (transfers) is placed on the surface of the skin, which is partially dehydrated by the loss of evaporation of the water, and therefore the lipid vesicles experience this "osmotic gradient" and they try to escape complete drying by moving along this gradient. They can achieve this only if they are deformable enough to pass through the narrow pores of the skin, like transfersomes that are composed of surfactant have more adequate rheological and hydration properties and is therefore responsible for their greater deformability. Some less deformable vesicles, such as liposomes are limited to the skin surface, where they are completely dehydrated and melted; therefore they have a lower penetrating power than transfersomes.[14]

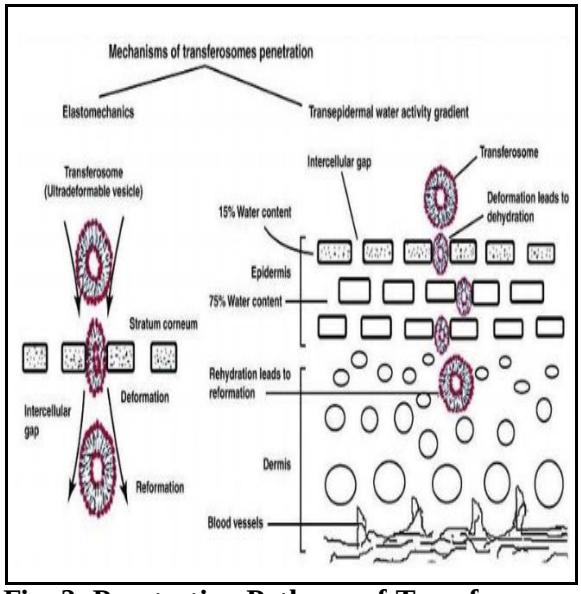

Fig. 2: Penetration Pathway of Transfersomes

Transfersomes are optimized in this regard and therefore obtain maximum flexibility and can fully exploit the transepidermal osmotic gradient (water concentration gradient) and overcome the skin penetration difficulty by squeezing themselves along the intracellular sealing lipids of stratum corneum. This extreme ability to deform enables transfersomes to percolate into skin through temporarily opened pores, through which normally water evaporated. These deformable vesicles after entering into medium of higher water content in deeper skin cells reversibly regain their shape. And since they are too large to enter blood vessels locally they arrive into blood circulation via lymphatic system.[6]

At present, the mechanism of enhancing the delivery of active substances in and across the skin is not very well known. However two mechanisms that have been proposed are:

1. Transfersomes act as drug carriers and remain intact after entering the skin.

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2. Transfersomes act as penetration enhancers, altering the highly organized intercellular lipids of the stratum corneum and thus facilitating the penetration of the drug molecule into and through the stratum corneum.

Recent studies have suggested that the penetration and permeation of vesicles through the skin are due to the combination of the two mechanisms. One of the two mechanisms prevails, depending on the nature of the active substance (lipophilic or hydrophilic) and the composition of the transfersomes.[15]

Propensity of Penetration:

Since transfersomes are too large to diffuse through the skin, they need to find their own route through the organ.

The magnitude of the driving force can then be calculated using the following formula:

Flow = Area × (Barrier) permeability × (Trans-barrier) force.

Hence flow of the lipid across the skin which is chemically driven, decreases drastically when the lipid in solution form is replaced by the same amount of suspension of lipid.[16]

Composition of Transfersome:

Main components of transfersomes are:

- Amphipathic agents or phospholipids such as phosphotidyl choline self assemble into a lipid bilayer in aqueous environment and closes to form vesicle.
- Bilayer softening agent such as surfactants namely Tween 80, Span 85, Span 80, Sodium cholate, Sodium deoxycholate etc. help in giving the vesicles their characteristic deformable and flexible property by softening the lipid bilayer.

The flexibility and permeability of transfersome membrane depends on the ratio of surfactant to edge activator used, which make transfersome ultra deformable.[17]

Other commonly used components required for transfersomes preparations are also given in Table 2.

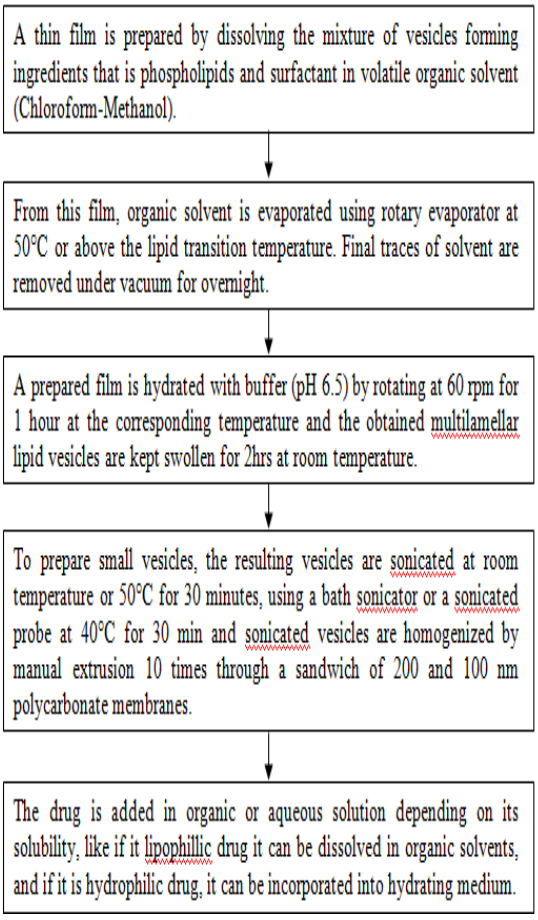
Table 2: Different Additives Used in Formulation of Transfersomes [18]

Class	Example	Uses
Phospholipids	Soya phosphatidyl choline Dipalmitoyl phosphatidyl choline Distearoyl phoshatidyl choline	Vesicles forming component
Surfactant/ Edge activators	Sodium cholate Sodium deoxycholate Tween-80 Span-80	For providing flexibility
Alcohol	Ethanol, Methanol, Isopropyl alcohol	As a solvent
Dye	Rhodamine-123 Rhodamine-DHPE Fluorescein-DHPE Nile-red	Used for confocal laser Scanning microscopy study
Buffering agent	Saline phosphate buffer (pH 6.4)	As a hydrating Medium

Method of Preparation of Transfersomes:

Rotary Film Evaporation Method [19]**:**

This method is also known as thin film hydration method and the procedure is shown below.



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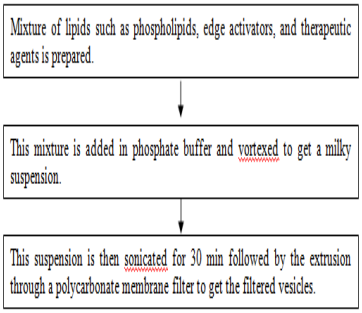
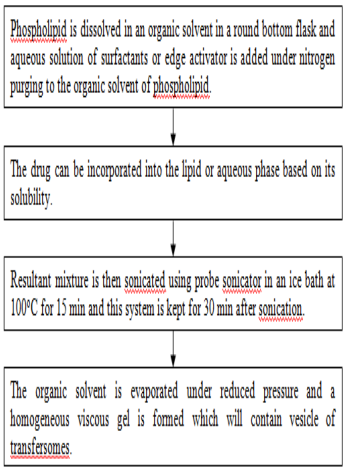
Vortexing Sonication Method [20]**:**



Reverse phase Evaporation Method [20]**:**



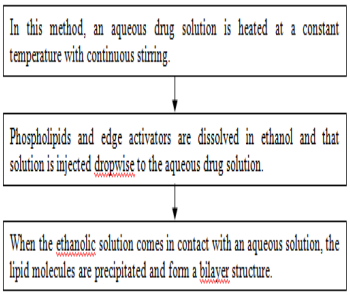
Aqueous Lipid Suspension Process [1]**:**

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

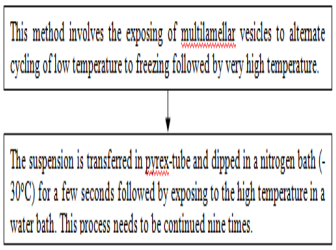
Suspension Homogenization Process [7]**:**

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

Ethanol Injection Method[20]**:**

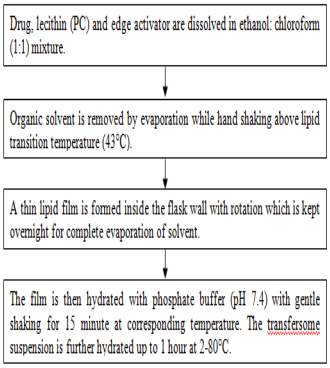


Freeze Thaw Method [20]**:**



Modified Hand Shaking Method [10,19]**:**

This method is also known as lipid film hydration technique and is described in fig.



Optimization Factors of Transfersomes Formulation [21,22]**:**

Various process variables that affect the preparation and properties of the transfersomes are:

pH:- As the pH affects the molecule ionization and consequently its interaction and entrapment efficiency, it's level should be adequate for achieving a balance between formulation properties and biological applications, including the administration route. The evaluation of pH is done by potentiometry.

Effect of Type of Edge Activator:- Vesicle size of transfersomes, entrapment efficiency, stability, deformability and permeation ability through the skin is affected by the amount and type of edge

activators used in the preparation. Gehanne A.S Awad et.al in his article suggested that entrapment efficiency of transfersomes depends on the HLB value of the surfactants like Span 85 (1.8), Span 80 (4.3), Tween 80 (15), Sodium deoxycholate and Sodium cholate (16.7). The affinity of these surfactants towards lipids has been seen to be of the order Span 85>Span 80>Tween 80>Sodium cholate/ Sodium deoxycholate, which suggests higher entrapment efficiency with Span 85 and Span 80.

Another correlation of edge activators and vesicle deformability has also been suggested in which deformability of vesicles depends on the chemical structure of these surfactants. Tween 80 showed highest deformability due to its highly flexible and non-bulky hydrocarbon chains.

Effect of Phospholipid to Edge Activator Ratio:- An optimized ratio of phosphatidylecholine and edge activator should be there, because this affects greatly the entrapment efficiency. Higher concentration of edge activators may decrease entrapment efficiency. Upon incorporation of edge activators in low concentration, growth in vesicle size occurred, whereas further increase in the content of edge activator may have led to pore formation in the bilayer and reduction in penetration through skin.

Effect of Total Lipid Concentration:- Various research findings indicated that the fraction of lipid taking part in encapsulation gets reduced upon increasing the total lipid concentration. Total lipid concentration used for formulation affects entrapment efficiency.

Effect of Method of Preparation:- Preparation method also influences the formulated transfersomes. Two widely used methods are namely vortexing and thin film hydration. Among these two, thin film hydration using rotary vacuum evaporator gave finer particles and better entrapment efficiency as compared to the other methods. This is due to the larger surface area obtained by the formation of a thin film which leads to complete hydration of vesicles and thus better vesicle characteristics. On the other hand, vortexing gives rise to aggregation of the lipids and thus sticking on the walls of the vial making hydration difficult. This gives a lumpy mass which sediments quickly upon storage.

Effect of Organic Solvents Used:- The choice of solvent used depends on the solubility of all the ingredients added and also on their compatibility with the solvents. Ideally, all the excipients should fully dissolve in the solvent and a clear transparent solution should be formed. The effect of organic solvent on solubility, film formation, hydration and stability of the so formed transfersomes is explained in the article by author Wen-sheng Zheng in preparation and quality assessment of itraconazole transfersomes. The results in the study suggested that the raw excipient dissolved in anhydrous ethanol and chloroform gave poor solubility and also non-uniform film formation. Flocculation and precipitation was also seen after hydration. On the other hand, use of a mixture of these two solvents in 1:1 ratio gave a clear transparent solution and better film forming ability and good stability after hydration.

Characterization of Transfersomes:

The characterisation of transfersomes can be done by on following parameters:

Vesicle Morphology [23]**:-**

(a) Vesicle Diameter: Determination of vesicle diameter can be done using photon correlation spectroscopy or dynamic light scattering (DLS) method. Samples are prepared in distilled water, filtered through a 0.2 mm membrane filter and diluted with filtered saline and then size measurement is done using photon correlation spectroscopy or dynamic light scattering (DLS) measurements. The stability of vesicle can be determined by assessing the size and structure of vesicles over time.

(b) Vesicle Shape and Type: The visualization of vesicle shape or its appearance can be carried out using TEM. They can also be visualized by phase contrast microscopy using optical microscopy method, without sonication. Dynamic light scattering technique can also be used.

(c) Vesicle Size, Size Distribution and Zeta Potential: To study the vesicular shape, Transmission electron microscopic (TEM) studies are used. The size of the vesicle, size distribution and zeta potential is generally determined by Dynamic Light Scattering

Method (DLS) using a computerized inspection system by Malvern Zetasizer.

No. of Vesicles per Cubic mm [24]**:-**

For optimizing the composition and other process variables, number of vesicles per cubic mm is an important parameter. In this non-sonicated transfersome formulations are diluted five times with 0.9% sodium chloride solution. For further study, haemocytometer and optical microscope can then be used. The numbers of transfersomes in 80 small squares are counted and calculated using the following formula:

$$\text{Total No. of Transfersomes mm}^3 = \frac{\text{Total No. of Vesicles Counted} \times \text{Dilution Factor} \times 4000}{\text{Total No. of Squares Counted}}$$

Entrapment Efficiency [25]**:-**

Entrapment efficiency is expressed as the amount of the drug entrapped in percent of drug added. This can be determined by first separation of the unentrapped drug using mini-column centrifugation method. After centrifugation, the vesicles are disrupted using 0.1% Triton X-100 or 50% n-propanol. The entrapment efficiency is expressed as:

Entrapment Efficiency = (Amount of drug entrapped/ Total amount of drug added) × 100

Drug Content [23]**:-**

One of the instrumental analytical methods which can be used for drug content determination is modified high performance liquid chromatography method (HPLC) method using a UV detector.

Confocal Scanning Laser Microscopy Study [25]**:-**

In conventional light microscopy and electron microscopy problem of fixation, sectioning and staining of the skin samples occurs. The structures to be examined are often incompatible with the corresponding processing techniques that give rise to misinterpretations, which can be minimized by means of 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:

- To study the penetration mechanism of transfersomes through the skin,
- To determine the histological organization of the skin (epidermal columns, interdigitation) and the shapes and architecture of the skin penetration pathways.
- To compare and differentiate the penetration mechanism of transfersomes with liposomes, niosomes and micelles.

Different fluorescence markers that can be used in CSLM study are as follows:

1. Fluorescein-DHPE:- N-(fluorescein-5-thiocarbamoyl)- 1,2- dihexadecanoyl-sn-glycero-3-phosphoethanolamine, triethyl-ammonium salt.
2. Rhodamine-DHPE:- N-(Lissamine Rhodamine B sulfonyl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine, triethylammonium salt.
3. NBD- PE:- (1, 2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine-N-(7-nitro-Benz- 2-oxa-1,3-diazol-4-yl) triethanolamine salt.
4. Nile red.

Degree of Deformability or Permeability Measurement [24]**:-**

The permeability study is one of the most important and unique parameter for characterization of transfersomes. The deformability study is performed using the pure water as standard, in which 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). After each pass, particle size and size distributions are noted by dynamic light scattering (DLS) measurements. The degree of deformability can be determined using the following formula, as reported by Berge Vanden et al.:

$$\mathbf{D} = J \times \left(\frac{rv}{rp}\right)^2$$

Where,

D = Deformability of vesicle membrane

J = The amount of the suspension extruded during 5min;

rv = The size of the vesicle;

rp = Pore size of the barrier or membrane.

Turbidity and Surface Charge Measurement [23]**:-**

(a) Turbidity: Turbidity of the any drug is measured in the form of aqueous solution using a nephelometer.

(b) Surface Charge and Charge Density: A zeta sizer is used to determine the surface charge and charge density.

Penetration Ability [23]**:-**

Fluorescence microscopy is generally used to evaluate penetration ability of transfersomes.

Occlusion Effect [24]**:-**

In case of traditional topical preparations, occlusion of skin is considered to be helpful for drug permeation, but the same proves to be detrimental for elastic vesicles. In transfersomes, hydrotaxis (movement in the direction) of water is the major driving force for permeation through the skin i.e. from its relatively dry surface to water rich deeper regions. Occlusion affects hydration forces as it prevents evaporation of water from skin hence it is important to study.

Physical Stability [23]**:-**

In this, the initial percentage of the drug entrapped in the formulation is determined and transfersomes are stored in sealed glass ampoules. The ampoules are placed at 4 ± 20°C (refrigeration), 25 ± 20°C (room temp), and 37 ± 20°C (body temp) for at least 3 months. Samples are taken from each ampoule and analyzed after 30 days to determine drug leakage. Percent drug loss is calculated by keeping the initial entrapment of drug as 100%.

In-vitro Drug Release [25]**:-**

In vitro drug release study is performed to determine the rate of permeation. The 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 drug release study, transfersome suspension is incubated at 32°C and samples are taken at different times with separation of free drug by mini column centrifugation. The amount of drug released is then calculated indirectly from the amount of drug entrapped at zero times as the initial amount (100% entrapped and 0% released).

In-vitro Skin Permeation Studies [24]**:-**

For this study, Modified Franz diffusion cell with a receiver compartment volume of 50 ml and effective diffusion area of 2.50 cm^2 is used. In vitro permeation study is performed using fresh abdominal skin of goat collected from slaughterhouse in phosphate buffer solution (pH 7.4). Abdominal hairs of skin are removed and the skin is hydrated in normal saline solution. From the skin, the adipose tissue layer is removed by rubbing with a cotton swab and is kept in isopropyl alcohol solution and stored at 0-4°C.

To perform the study, treated skin should be mounted horizontally on the receptor compartment with the stratum corneum side facing upwards towards the donor compartment of Franz diffusion cell. The receptor compartment is filled with 50ml of phosphate buffer (pH 7.4) saline maintained at 37 ± 0.50°C and stirred by a magnetic bar at 100rpm. Formulation (equivalent to 10 mg drug) is placed on the skin with covering the top of the diffusion cell. At adequate time intervals, 1 ml aliquots from receptor medium is withdrawn and immediately replaced by an equal volume of fresh phosphate buffers (pH 7.4) to maintain sink conditions. The sample analysis is done by any of instrumental analytical technique after considering the correction factors for each aliquot during calculation of release profile.

Skin Deposition Studies of Optimized Formulation [23]**:-**

The ability of transfersomes to help in retaining the encapsulated drug within the skin (depot-effect) in comparison to liposomes, plain drug and commercial product is investigated by this method. After the end of permeation study (at the end of 24 h), the skin surface is washed five times with a solution containing ethanol:PBS (pH 7.4) in the ratio 1:1 and then with water to remove excess drug from skin surface. The skin is then cut into small pieces and tissue is further homogenized with approx 100ml of ethanol:buffer solution pH 7.4 (1:1) at 37±0.5°C and left for 6hr at room temperature. After 5min shaking and centrifuging it for 5 min at 5000 rpm, the filtrate is removed and drug content is analyzed using appropriate dilutions with phosphate buffer solution (pH 7.4). The result is compared, using a student's t test, with that of the control. (ijrpns, rjpdft)

Tape Stripping Method [13,14]**:-**

This technique is a widely accepted and used to examine the localization and distribution of substances within the stratum corneum. It is the simplest method to reduce the barrier imposed by the stratum corneum by removing it. In this technique, an adhesive tape removes a layer of

corneocytes, therefore *in vivo* removal of the stratum corneum by tape stripping is performed by the repeated application of adhesive tapes to the skin's surface. This can be used to investigate stratum corneum cohesion *in vivo* by quantifying the amount of stratum corneum removed. Tape stripping method can be studied in combination with electron microscopy and FT-IR. There are different parameters that can affect the quantity of stratum corneum removed by a piece of tape, and these include tape stripping mode, skin hydration, cohesion between cells, the body site and inter-individual differences.

Stability Studies [25]**:-**

Stability of the transfersomes is determined by TEM visualization at 4°C and 37°C. DLS size measurement can also be used at different time intervals (30, 45, and 60 days), following vesicles preparation. The percent drug loss is calculated by considering the initial entrapment 100%.

Applications:

Delivery of Proteins and Peptides:

For transportation of larger biogenic molecules of body such as proteins and peptides, transfersomes are being widely used as a carrier otherwise given through oral routes such molecules get degraded in GIT. Because of this reason proteins and peptides still have to be introduced into the body through injections but now transfersomes have proven to be a suitable approach as they also provide bioavailability somewhat similar to subcutaneous injections of same protein suspension.

Several proteins have been administered by preparing transfersomes e.g. bovine serum albumin, after the repeated epicutaneous application, the transferosomal preparations induced strong immune response. Gap junction proteins loaded in transfersomes also elicited antigen specific antibody titer equivalent to subcutaneous route.[26]

Delivery of Anti-cancer Drugs:

Transfersomes have also been tried for delivery of anti-cancer agents like methotraxate as they are especially suitable to be used as a carrier for treatment of skin cancer. The obtained results were favorable and this provided a new approach for treatment especially of skin cancer. Anti breast cancer agent Tamoxifen (TAM) is delivered by transfersomes through the skin most efficiently and also accelerated the growth of murine uteri, where it act as an anti-oestrogen, even at low dose as 0.1-0.2 mg/kg/day.[21]

Delivery of Herbal Drugs:

Due to the property of transfersome to supply nutrients locally by penetrating the stratum corneum, transfersomes of capsaicin have been prepared by Xiao-Ying et al., showing improved absorption through the topical route when compared to pure capsaicin.[27]

Transfersomes are also used to increase the skin penetration of certain phyto-constituents like capsaicin and colchicine and along with these effects also increase the entrapment efficiency of certain phytoconstituents like vincristine.[12]

Delivery of Insulin:

Transfersomes have been used to deliver insulin to the systemic circulation in therapeutic amounts equivalent to subcutaneous injection. Cevc et al. reported about the study for insulin delivery from transfersomes composed of phosphatidylcholine incorporating sodium cholate and compared with conventional liposomes and mixed micelles applied to the skin of both mice and humans. There was a 30-min lag time relative to a subcutaneous injection of the same formulation, but overall efficacy of delivery was comparable.[28]

Delivery of Interferons:

Transferosomes have also been used as a carrier for interferons, for example leukocytic derived interferone-α (INF-α) is a naturally occurring protein having antiviral, antiproliferive and some immunomodulatory effects. Hafer et al studied transferosmes loaded with immunomoduolators, interleukin-2 and interferone-α for potential transdermal application. Studies reported that delivery of IL-2 and INF- α trapped by transferosomes occurred in sufficient concentration for immunotherapy.[26]

Transdermal Immunization:

Ultradeformable vesicles due to their capability of delivering larger molecules can also be exploited for topical delivery of vaccines. Cationic transfersomes of Plasmid DNA encoding Hepatitis-B surface antigen (HBs-Ag) are also utilized for topical immunization and showed significantly higher HBs-Ag antibody titer and cytokinins level.[29] Ex vivo cellular uptake and fluorescence microscopy studies demonstrated

skin permeation, biodistribution and efficient delivery of antigens to the immunocompetent Langerhan's cells and lymphatics.[28]

Controlled Release and Solubility Enhancement:

As drug delivery system, transferosomes have the potential to provide controlled release of the administered drug and increasing the stability of labile drugs. In one of the previous study, transfersomal gel of metronidazole was prepared and the results obtained proved the transfersomal gel as a promising candidate for transdermal delivery with targeted and prolonged release of a drug and also enhanced the skin permeation of many drugs.[30]

To explore solubility enhancement activity of poorly aqueous soluble drugs, transfersomes of several drugs have been made in various studies. Marwa H et al. formulated transfersomal vesicles as a transdermal drug delivery system for poorly soluble drug, Nystatin. This research suggested that nystatin loaded transfersomes can be potentially used as a transdermal drug delivery system for solubility enhancement.[31]

Delivery of Corticosteroids:

Potential of transferosomes have been used for the delivery of corticosteroids as they improve site specificity and overall drug safety margin of corticosteroid delivery into skin by optimizing the epicutaneously administered drug dose. Transferosomes based corticosteroids are biologically active at dose several times lower than the currently used formulation for the treatment of skin diseases.[32]

Transfersomes for Delivery of Anesthetics and NSAID's:

In suspension of highly deformable vesicles, anesthetics induce a topical anesthesia under appropriate conditions, with less than 10 min. From transfersomes maximum resulting pain insensitivity obtained is nearly as strong (80%) as that of a comparable subcutaneous bolus injection. The effect of transfersomal anesthetics last longer than parenteral route.[33]

NSAIDS are associated with number of GI side effects which can be overcome by using ultra-deformable vesicles. Studies have been carried out on Diclofenac and Ketoprofen. Transfersomal formulation of Ketoprofen gained marketing approval by the Swiss regulatory agency (SwissMedic) in 2007; the product is expected to be marketed under the trademark Diractin. These studies were done to enhance the transdermal penetration of a poorly water soluble NASID, piroxicam, by encapsulating it into a nano-carrier namely transfersomes. Further therapeutic products based on the transfersome technology, according to IDEA AG, are in clinical development.[34]

Peripheral Drug Targeting:

Minimal carrier associated drug clearance of transfersomes helps in targeting to peripheral blood vessels in the subcutaneous tissue. Endothelial cells tight junctions restrict the vesicles in peripheral tissues.[33]

Conclusion

From literatures, it is evident that transfersomes being ultra deformable vesicles can deliver both small as well as larger molecules efficiently by overcoming various problems of skin permeation. These not only assist in transportation of substances through skin but can also be used for solubility enhancement of poorly soluble drugs. Their exceptional quality of modifying shape in response to external stress has made them a suitable candidate for sustained, controlled and targeted drug delivery. Because of numerous characteristics, transfersomes are gaining interest in market and ensuring bright future as transdermal drug delivery of drugs. The purpose of this review is to compile the maximum available knowledge from the articles that gave promising results so as to pave the way for further development of similar transfersomal preparations.

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