



Received on 19 June, 2013; received in revised form, 19 August, 2013; accepted, 21 October, 2013; published 01 November, 2013

TRANSFEROSOME: LATEST UPDATES

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Keywords:

Transferosomes, Pharmacokinetic, Analgesic, Anesthetic, Corticosteroids, Lecithin, Stratum Corneum, Surfactant, Vesicles, Transdermal Drug Delivery

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ABSTRACT: Poor patient compliance is a frequent problem in daily clinical practice. The unfavorable pharmacokinetic of the drug, the inconveniences of the standard form 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 transferosomes membrane, which are adaptable to ambient stress allow the ultra-deformable transferosomes to change its membrane composition locally and reversibly, when it is pressed against or attracted into narrow pore. Transferosomes possess an infrastructure consisting of hydrophobic and hydrophilic moieties together and as a result can accommodate drug molecules with wide range of solubility. Transferosomes 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.

INTRODUCTION: Liposomal as well as niosomal systems, are not suitable for transdermal delivery, because of their poor skin permeability, breaking of vesicles, leakage of drug, aggregation, and fusion of vesicles. To overcome these problems, a new type of carrier system called "transferosome", has recently been introduced, which is capable of transdermal delivery of low as well as high molecular weight drugs. Transferosomes are specially optimized, ultra-deformable (ultraflexible) lipid supra molecular aggregates, which are able to penetrate the mammalian skin intact as shown in **fig. 1**.

Each transferosome consists of at least one inner aqueous compartment as shown in **fig. 2**, which is surrounded by a lipid bilayer with specially tailored properties, due to the incorporation of "edge activators" into the vesicular membrane. Surfactants such as sodium cholate, sodium deoxycholate, span 80, and tween 80 have been used as edge activators. These novel carriers are applied in the form of semi-dilute suspension, without occlusion.

Due to their deformability, transferosomes are good candidates for the non-invasive delivery of small, medium, and large sized drugs. Transferosomes are vesicles composed by phospholipids as the main ingredient (soya phosphatidylcholine, egg phosphatidylcholine, dipalmityl phosphatidylcholine, etc), 10- 25% surfactants for providing flexibility (sodium cholate, tween 80, span-80), 3-10% alcohol as a

	<p style="text-align: center;">DOI: 10.13040/IJPSR.0975-8232.4(11).4125-32</p>
	<p style="text-align: center;">Article can be accessed online on: www.ijpsr.com</p>
<p>DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.4(11).4125-32</p>	

solvent (ethanol, methanol) and hydrating medium consisting of saline phosphate buffer (pH 6.5-7). Transferosome is a term registered as a trademark by the German company IDEA AG, and used by it to refer to its drug delivery technology. The name means “carrying body”, and is derived from the

Latin word 'transfere', meaning 'to carry across', and the Greek word 'soma', for a 'body'. A Transferosome carrier is an artificial vesicle designed to be like a cell vesicle or a cell engaged in exocytosis, and thus suitable for controlled and, potentially targeted, drug delivery¹⁻².

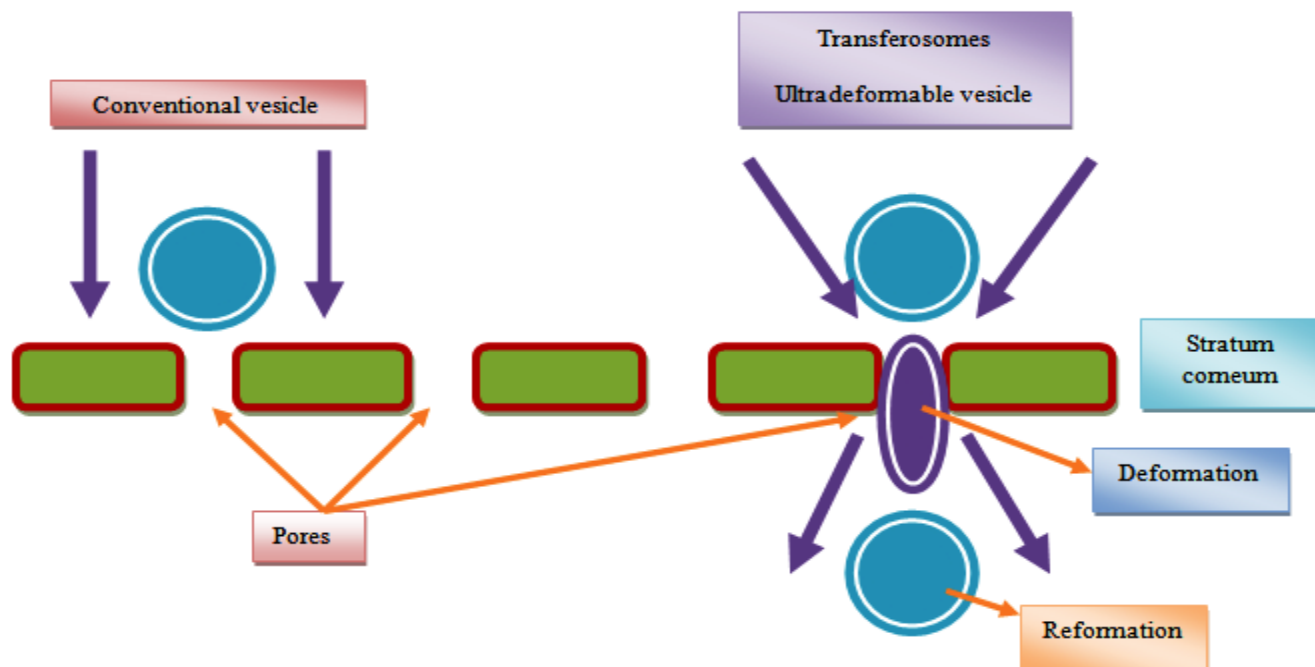


FIG. 1: SCHEMATIC DIAGRAM DESCRIBING INTERACTION OF THE TRANSFEROSOMES WITH SKIN TISSUES

Advantages³⁻⁵: Transferosomes have greater advantages. These shows greater permeation of the drugs through the skin because of its flexible membranes

- Transferosomes consists of hydrophobic and hydrophilic moieties together and as a result can accommodate drug molecules with wide range of solubility.
- These serves as carrier for both small and large molecular weight drugs e.g. analgesic, anesthetic, corticosteroids, sex hormone, anticancer, insulin, gap junction protein, and albumin.
- In transferosomes percentage of the drug entrapment is more, in case of lipophilic drug near to 90%.
- They protect the entrapped drug from atmospheric degradation.
- These are biocompatible and biodegradable as they are prepared with natural phospholipids.

- By selecting the suitable composition and administration mode, we can have site specific therapy.
- They can be used for both systemic as well as topical delivery of drug.
- They act as depot, releasing their contents slowly and gradually.
- Easy to scale up, as procedure is simple.

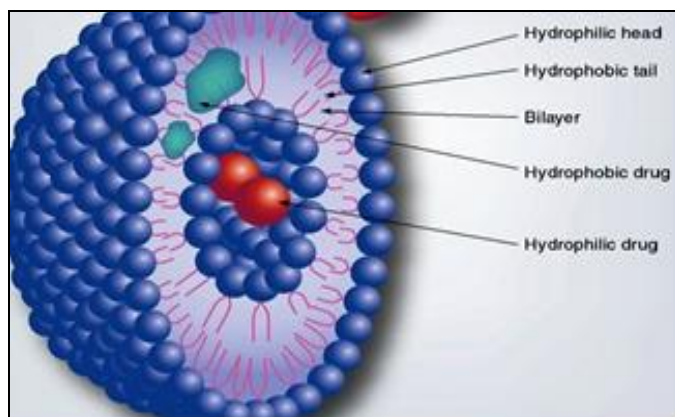


FIG. 2: DIAGRAM OF TRANSFEROSOME

Limitations⁵⁻⁷:

- Like liposomes, transferosomes have certain limitations
- Transferosomes are chemically unstable because of their oxidative degradation.
- Lack of purity of the natural phospholipids comes in the way of adoption of transferosomes as drug delivery vehicles.
- Transferosomes formulations are expensive to prepare.

Application of Transferosomes: Transferosomes have been widely used as a carrier for the transport of proteins and peptides. Proteins and peptide are large biogenic molecules which are very difficult to transport into the body, when given orally they are completely degraded in the GI tract. These are the reasons why these peptides and proteins are still have to be introduced into the body through injections. Various approaches have been developed to improve these situations. The bioavailability obtained from transferosomes is somewhat similar to that resulting from subcutaneous injection of the same protein suspension. The transferosomal preparations of this protein also induced strong immune response after the repeated epicutaneous application, for example the adjuvant immunogenic bovine serum albumin in transferosomes, after several dermal challenges is as active immunologically as is the corresponding injected proteo-transferosomes preparations.

Delivery of insulin by transferosomes is the successful means of noninvasive therapeutic use of such large molecular weight drugs on the skin. Insulin is generally administered by subcutaneous route that is inconvenient. Encapsulation of insulin into transferosomes (transfersulin) overcomes these entire problems. After transfersulin application on the intact skin, the first sign of systemic hypoglycemia are observed after 90 to 180 min, depending on the specific carrier composition.

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. Transferosomes as drug delivery systems have the potential for providing controlled release of the administered drug and increasing the stability of labile drugs. Hafer *et al* studied the formulation of interleukin-2 and interferone- α containing transferosomes for potential transdermal application .they reported delivery of IL-2 and INF- α trapped by transferosomes in sufficient concentration for immunotherapy.

Another most important application of transferosomes is transdermal immunization using transferosomes loded with soluble protein like integral membrane protein, human serum albumin, gap junction protein. These approach offers at least two advantages, first they are applicable without injection and second, they give rise to rather high titer and possibly, to relatively high IgA levels.

Transferosomes have also used for the delivery of corticosteroids. Transferosomes improves the site specificity and overall drug safety of corticosteroid delivery into skin by optimizing the epicutaneously administered drug dose. Transferosomes based cortiosteroids are biologically active at dose several times lower than the currently used formulation for the treatment of skin diseases.

Application of anesthetics in the suspension of highly deformable vesicles, transferosomes, induces a topical anesthesia, under appropriate conditions, with less than 10 min. Maximum resulting pain insensitivity is nearly as strong (80%) as that of a comparable subcutaneous bolus injection, but the effect of transferosomal anesthetics last longer. Transferosomes has also been used for the topical analgesics, anaesthetics agents, NSAIDS and anti-cancer agents.

Composition and Mechanism: A transferosome is a self-adaptable and optimized mixed lipid aggregate. The surfactant molecules act as “edge activators”, conferring ultra-deformability on the transfero-somes, which reportedly allows them to squeeze through channels in the stratum corneum that are less than one-tenth the diameter of the transferosome. According to their inventors, where liposomes are too large to pass through pores of less than 50 nm in size, transferosomes up to 500 nm can squeeze through to penetrate the stratum corneum barrier spontaneously¹⁴.

They suggest that the driving force for penetration into the skin is the “transdermal gradient” caused by the difference in water content between the relatively dehydrated skin surface (approximately 20% water) and the aqueous viable epidermis (close to 100%).

Deformability of transferosomes is achieved by using surface active agent in the proper ratio. The concentration of surface active agent is crucial in the formulation of transferosomes because at sublytic concentration these agents provide flexibility to vesicle membranes and at higher concentration cause destruction of vesicles¹⁵. The resulting flexibility of transferosomal membrane minimizes the risk of complete vesicle rupture in the skin and allows the ultra-deformable transferosomes to change their membrane composition locally and reversibly, when they are pressed against or attracted into a narrow pore. This dramatically lowers the energetic cost of membrane deformation and permits the resulting highly flexible particles first to enter and then pass through the pores rapidly and efficiently.

The carrier aggregate is composed of at least one amphiphilic (such as phosphatidylcholine)¹⁶ which in aqueous solvent self resembles into lipid bilayer that closes into a simple lipid vesicle. By addition of at least one bilayer softening component (such as a biocompatible surfactant or an amphiphilic drug), lipid bilayer flexibility and permeation are greatly increased. Thus, by optimizing the resulting flexibility and permeability, the transferosome vesicles can adapt to their ambient shape easily and rapidly. Thus, they can also adjust the local concentration of each bilayer component to the local stress experienced by the bilayer. The basic organization of these vesicles is broadly similar to liposomes¹⁷. But the transferosomes differ from the conventional vesicles primarily by their softer, more deformable, and better adjustable artificial membrane.

Another beneficial consequence of strong bilayer deformability is the increased transferosome ability to bind and retain water. An ultradeformable and highly hydrophilic vesicle always seeks to avoid dehydration; this may involve a transport process related to, but not identical with, forward osmosis. For example, a transferosome vesicle applied on an open biological surface, such as non-occluded skin,

tends to penetrate its barrier and migrate into the water-rich deeper strata to secure its adequate hydration. Barrier penetration involves reversible bilayer deformation, but must not compromise unacceptably either the vesicle integrity or the barrier properties for the underlying hydration affinity and gradient to remain in place. Since it is too large to diffuse through the skin, the transferosome needs to find and enforce its own route through the organ.

The transferosomes usage in drug delivery consequently relies on the carrier's ability to widen and overcome the hydrophilic pores in the skin or some other barrier. The subsequent, gradual agent release from the drug carrier allows the drug molecules to diffuse and finally bind to their target. Drug transport to an intracellular action site may also involve the carrier's lipid bilayer fusion with the cell membrane, unless the vesicle is taken up actively by the cell in the process called endocytosis^{1, 7, 11}.

Mechanism of penetration of Transferosomes:

Transferosomes, when applied under suitable condition, can transfer 0.1 mg of lipid per hour and square centimeter area across the intact skin. This value is substantially higher than that 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. This osmotic gradient that 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)^{14, 17, 18}.

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 favorable interaction between the hydrophilic lipid residues and their proximal water. Thus, most lipid bilayers spontaneously resist an induced dehydration. 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.

So, when lipid suspension (transferosome) is placed on the skin surface that is partly dehydrated by the water evaporation loss, the lipid vesicles feel this “osmotic gradient” and try to escape complete drying by moving along this gradient. They can only achieve this if they are sufficiently deformable to pass through the narrow pores in the skin because transferosomes composed of surfactant have more suitable rheological and hydration properties than that responsible for their greater deformability; 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 the transferosome.

Transferosomes are optimized in this respect and thus attain maximum flexibility, so they can take full advantage of the transepidermal osmotic gradient (water concentration gradient). Transferosomes overcome the skin penetration difficulty by squeezing themselves along the intracellular sealing lipids of stratum corneum¹⁹.

At present, the mechanism of enhancing the delivery of active substances in and across the skin is not very well known. Two mechanisms of action have been proposed²⁰:

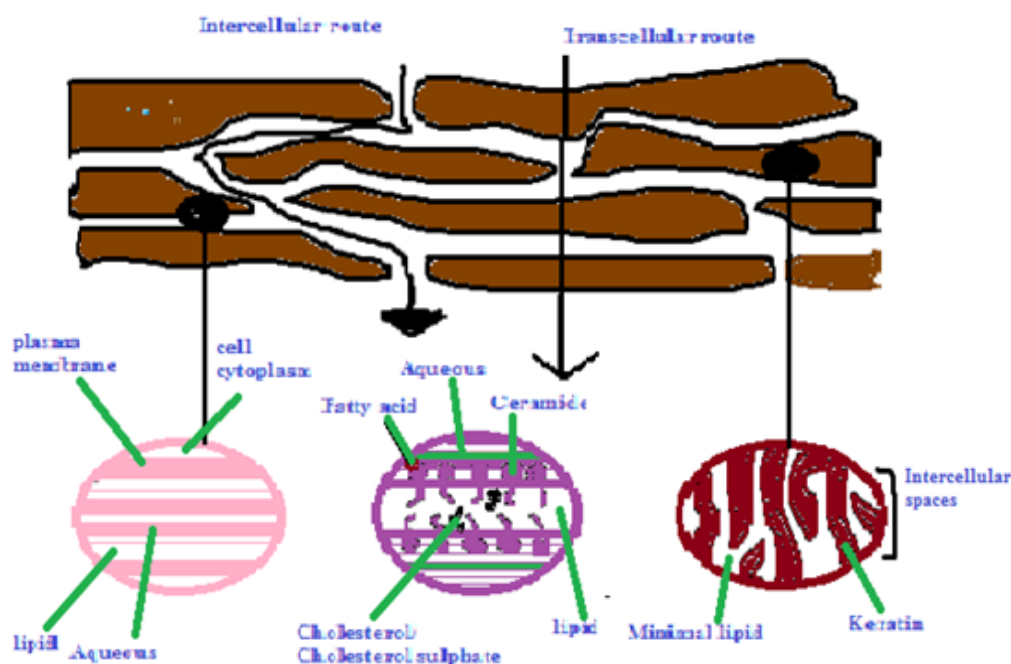


FIG. 3: MECHANISM OF PENETRATION OF TRANSFEROSOMES

- 1) Transferosomes act as drug vectors, remaining intact after entering the skin.
- 2) Transferosomes act as penetration enhancers, disrupting the highly organized intercellular lipids from stratum corneum, and therefore facilitating the drug molecule penetration in and across the stratum corneum.

Cevc and coworkers proposed the first mechanism, suggesting that deformable liposomes penetrate the stratum corneum because of the transdermal hydration gradient normally existing in the skin, and then cross the epidermis, and enter the systemic circulation.

The recent studies propose that the penetration and permeation of the vesicles across the skin are due to the combination of the two mechanisms. Depending on the nature of the active substance (lipophilic or hydrophilic) and the composition of the transferosomes, one of the two mechanisms prevails²¹.

Pathways of Transferosomes penetration²²:

1. Through stratum corneum
2. Transfollicular
3. Through sweat glands

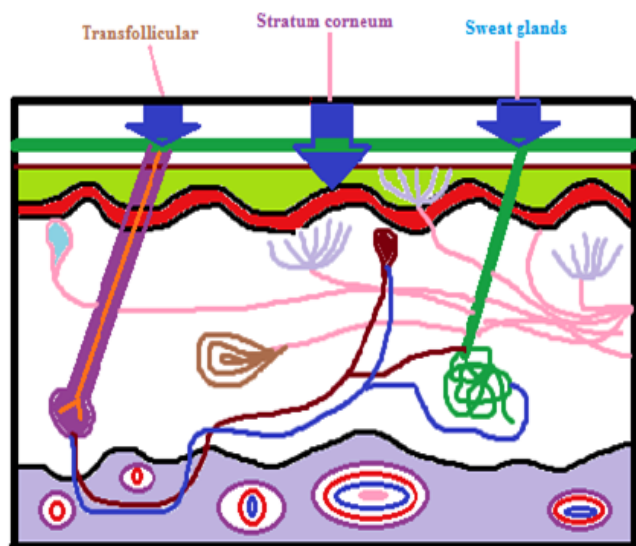


FIG. 4: PATHWAYS OF TRANSFEROSOMES PENETRATION

TABLE 1: DIFFERENT ADDITIVES USED IN FORMULATION OF TRANSFEROSOMES⁹⁻¹⁴

Class	Example	Uses
Phospholipids	Soya phosphatidyl choline Dipalmitoyl phosphatidyl choline Distearoyl phosphatidyl choline	Vesicles forming component
Surfactant	Sod. Cholate Sod. deoxycholate tween-80 Span-80	For providing flexibility
Alcohol	Ethanol	As a solvent
Dye	Rhodamine-123 Rhodamine-DHPE Fluorescein-DHPE Nile-red	For CSLM study
Buffering agent	Saline phosphate buffer (pH 6.4)	As a hydrating medium

The deposited lipid films were hydrated with buffer (pH 6.5) by rotation at 60 rpm min⁻¹ for 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 500°C for 30 min. bath sonicator or probe sonicated at 40°C 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.

Stability of Transferosomes: Transferosomes are chemically unstable because of their predisposition to oxidative degradation. Purity of natural phospholipids is another criterion militating against adoption of transferosomes as drug delivery vehicles. Transferosome formulations are expensive.

MATERIALS AND METHODS: Materials commonly used for the preparation of transferosomes are summarized in **Table 1**.

Method of preparation²²⁻³³: Methods of preparation of transferosomes 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 500°C for dipalmitoyl phosphatidyl choline) using rotary evaporator. Final traces of solvent were removed under vacuum for overnight.

Characterization of Transferosomes²²⁻³³:

- 1. Entrapment efficiency:** Entrapment efficiency was determined by first separation of untrapped drug by the use of mini-column centrifugation method. After centrifugation, the vesicle was disrupted using 0.1% Triton X-100 or 50% n-propanol and then followed by suitable analytical technique to determine the entrapped drugs.
- 2. 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 then size measurement done by using photon correlation spectroscopy or dynamic light scattering measurements.

3. **Confocal Scanning Laser Microscopy (CSLM) study:** In this technique, lipophilic fluorescence markers are incorporated into the transferosomes and the light emitted by these markers are used for the investigation of mechanism of penetration of transferosomes across the skin, for determining histological organization of the skin and for comparison and differentiation of the mechanism of penetration of transferosomes with liposomes, niosomes and micelles.
 4. **Degree of Deformability or Permeability measurement:** The deformability study is done against the pure water as standard. Transferosomes preparation is passed through a large number of pores of known size through a sandwich of different micropores filters with pore diameter between 50 nm and 400 nm, depending on the starting transferosomes suspension. Particle size and size distribution are noted after each pass by dynamic light scattering (DLS) measurements.
 5. ***In vitro* drug release:** The information from in-vitro studies are used to optimize the formulation before more expensive *in vivo* studies is performed. For determining *in vitro* drug release, beaker method is used in which transferosomes suspension is incubated at 32°C using cellophane membrane and the samples are taken at different times and then detected by various analytical techniques (U.V., HPLC, HPTLC) and the free drug is separated by minicolumn centrifugation, then the amount of drug release is calculated.
 6. **Vesicle shape and type:** Transferosomes vesicles can be visualized by TEM, with an accelerating voltage of 100 kv. Transferosomes vesicles can be visualized without sonication by phase contrast microscopy by using an optical microscope.
 7. **Number of vesicle per cubic mm:** This is an important parameter for optimizing the composition and other process variables. Transferosome formulations (without sonication) can be diluted five times with 0.9% of sodium chloride solution and studied with optical microscopy by using haemocytometer.
 8. **Penetration ability:** Penetration ability of transferosomes can be evaluated using fluorescence microscopy.
 9. **Turbidity measurement:** Turbidity of drug in aqueous solution can be measured using nephelometer.
 10. **Surface charge and charge density:** Surface charge and charge density of transferosomes can be determined using zetasizer.
- CONCLUSION:** The use of the transdermal route has been well established in the past, and because of its inherent advantages, new methods for transdermal delivery are continuously being developed. The introduction of ultradeformable vesicles, transferosomes, will thus surely become an important step in re launching the researches regarding the use of vesicles as transdermal drug delivery systems.
- In comparison to other transdermal delivery systems, the use of elastic vesicles has certain advantages: They allow enhanced permeation of drug through skin; their composition is safe and the components are approved for pharmaceutical and cosmetic use; they can increase the transdermal flux, prolonging the release and improving the site specificity of bioactive molecules; they can accommodate drug molecules with a wide range of solubility.
- Hence, enhanced delivery of bioactive molecules through the skin by means of an ultradeformable vesicular carrier opens new challenges and opportunities for the development of novel improved therapies.
- Thus, it could be concluded that the new ultra flexible drug carrier (transferosome) can overcome all the problems associated with the transdermal delivery as transferosomes itself are specially optimized vesicles having the capability of responding to an external stress by rapid and energetically inexpensive shape transformations.
- ACKNOWLEDGEMENT:** The authors are grateful to our respected Mr. Manohar Reddy and Mr. Lakshmana Reddy, the Chairmen of Trinity and Jangaon College of Pharmaceutical Sciences for providing necessary facilities to do this work.

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How to cite this article:

Kumar VD, Saraswathi B and Kanth NP: Transferosome: Latest Updates. *Int J Pharm Sci Res* 2013; 4(11): 4125-32. doi: 10.13040/IJPSR. 0975-8232.4(11).4125-32

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