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Bicosome: A versatile technology in biomedicine and dermopharmacy

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ABSTRACT

Bicosomes opened a new chapter in vesicular drug delivery system which immensely contributed to the development of pharmaceutical research. They are defined as bicelles encapsulated in liposomal vesicular structure. One of the peculiar characteristics of bicosomes lies in its distinctive ability to maintain the morphology without any structural changes. They preserve and isolate bicelles from environments with high water contents. This drug delivery system has remarkable stability over temperature changes, in highly diluted media and also an effective permeability modulator for an effective transdermal drug delivery system. This vesicular system becomes a new application for the delivery of the drug through the skin. They are prepared by thin film hydration technique. Unique structures of bicosomes are small enough to pass through stratum corneum of the skin without any damage to tissues and cause structural transformation. This helps to hold the bicosomes between the skin layers and allows slow drug delivery. The capacity of these systems to regenerate skin barrier function and target specific action on skin layers finds its usefulness in the dermatological field. Thus bicosome technology is a versatile platform that can be applied in different skin disorders and appear to be smart nanosystems with great potential in biomedicine and dermopharmacy.

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INTRODUCTION

Bicosome opens a new chapter in a transdermal drug delivery system which is a challenge in pharmaceutical research. Skin being the most accessible and extensive organ of the body, it almost covers 2m^2 of the body's surface area and receives one-third of the blood circulating throughout the body (Nikhil *et al.,* 2011). Through passive diffusion alone the first generation small, lipophilic uncharged molecule were delivered to attain the therapeutic range, and most of the TDS belong to this class (William *et al.,* 2004). The second generation TDS products use additional force for drug delivery system. The new employing techniques such as microneedles and electrodeposition of macromolecules aim its effect towards the stratum corneum. (Prausnitz *et al.,* 2008). Finally, the fourth generation of TDS which includes formulations such as nanoparticles, microparticles, microspheres and liposomes (Shilakari *et al.,* 2013). The usage of the bicellar system in transdermal drug delivery made a phenomenal change in treating various diseases. These bicelles are defined as phospholipid aggregates which is either short chain (dihexanoyl PC) or long chain (dimyristoyl phosphatidylcholine), also a versatile class of model membrane which has an ability to align in the magnetic field (John *et al.,* 2005). The bicellar system is widely used for the solid-state NMR studies, in the system membrane models in diverse conformational studies of proteins and membrane peptides (Alexandre *et al.,* 2002). Depending on

the experimental molar ratio of the two lipids, the particle size is thought to range from 10-100 nm (Sanders *et al.,* 1992). Bicelles and micelles share some common traits like optically transparent, effectively monodispersed, non-compartmentalized. But bicelles has some added advantages over micelles, such as that it has low detergent content, maintain some key bilayer properties, and it is easier to achieve homogenous mixing than in lipid vesicles (Sanders *et al.,* 1998). Bicelles that will spontaneously be aligning in a magnetic field is having the phospholipid molar ratio between the long chain, and the short chain is 2.8-6.5, and their bilayer plane is parallel to the magnetic field (Glover *et al.,* 2001). The bicellar system is nowadays largely displacing the lipid-based liposomes and surfactant based micellar system as a model for evaluating membrane proteins due to their remarkable ability to mimic some membrane proteins (Cavagnero *et al.,* 1999).

Transdermal drug delivery system

Advantages

- It reduces the drug to be administered since it eliminates hepatic first pass metabolism.
- Highly useful for drugs which are bioavailable in GI tract.
- TDS is self-administrable by patient itself, and it is also pain-free.
- It maintains drug plasma concentration by eliminating frequent dose administration which is associated with oral and injections (Scheuplein,1965).
- It is a highly beneficial system in long term therapy of smoking cessation and chronic pain therapy, and the patient shows more compliance.
- It is useful for hepato- compromised patients (Misra,1997)
- Possible adverse drug reactions, intermittent dosing and therapeutic failures can be avoided.
- Drugs which are having poor oral availability, short half-life and narrow therapeutic window are effective through this route of administration (Barry *et al.,* 1983).
- Gastric and intestinal fluids do not interfere with bioavailability (Misra .1990).

Limitations

- Skin barrier changes from the site of application, age and individuals.
- Only smaller molecules having a size below 800-1000 Daltons can be administered (Harpin *et al.,* 1983).
- Absorption of larger molecules are difficult and desired therapeutic value may not be reached.
- Only lipophilic drugs are absorbed from the skin and drugs which are hydrophilic have lower penetration through the skin (Zhou *et al.,* 1997).
- The main disadvantage of TDS is local irritation at the site of application due to the drug, adhesive and other additives in the patch (Chien,1983).

Human skin

Human skin is a multi-layered, outermost, largest and most accessible organ in the body which constitutes about 8% of total body weight. It provides the largest interface between the body and the external environment (Scheuplein *et al.,* 1971). Cells, fibres, veins, capillaries, nerves and numerous small hair follicles make the skin a multi-layered structure. One of the peculiar function of the skin is to act as a physical and chemical barrier which protect our body from the entry of harmful chemicals, bacteria, virus and ultraviolet rays (Montagna, 1961). Flexibility and toughness of the skin make it resistant to friction and wound. The presence of nerves and nerve endings allow acting as one of the Important sensory organs. Maintenance of body temperature is mediated through the skin by a number of physiological activities like blood flow and sweating. It also takes part in excretion of harmful chemicals produced from intestine and liver through sweat and skin lipids. Age, race, gender influence the skin function, flexibility and toughness lose withthe age (McKenzie *et al.,* 1962)

Structure of skin

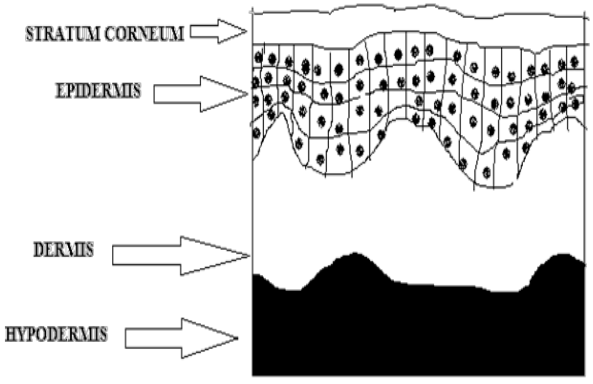

Figure 1: Skin structure

Human skin is a multi-layered and most accessible organ in the body. It allows various chemicals to pass through it and produce action both systemically and locally. It protects the internal organs from external influences, regulation of temperature and sensation. Skin is made up of three tissue layers: epidermis, dermis,

subcutaneous fat tissue (figure1and table1) (MachetL *et al.,* 2002).

Epidermis

This layer is stratified, squamous, keratinised and responsible for the evaluation of barrier function (keratinocytes). The five main sub-layers of the epidermis are stratum corneum, stratum basale, stratum spinosum, stratum granulosum, stratum lucidum. Veins and capillaries are not present in this layer. No blood flow through this layer (LampeMA *et al.,* 1983). Its composition allowsto keeping water within the body and foreign substances out. Therefore this layer is most important in TDS. A normal drug transfer across this layer is by passive diffusion, which is a slow process (Menon *et al.,* 1997).

Skin permeation and skin irritation

The composite structure of the skin barrier stated above helps in the permeation process. This structure is pierced by 40-70 hair follicles and 200-250 sweat glands, which are potential diffusion shunt (Kumar *et al.,* 2010). Water soluble substances are able to penetrate the skin in a very faster rate through this skin appendages than through the intact area of stratum corneum. But the trans appendageal route of absorption gives very limited contribution to the kinetic profile of skin permeation. The dosage form and extent of absorption of the drug from the site of action is the reason for desired therapeutic action and adverse effect in our body (Dhanalakshmi *et al.,* 2106).

Skin irritation adds to the potential side effects of this type of drug delivery. Different endogenous and exogenous factors seem to control the skin pH, the range of 5.4-5.9 which plays a major role in defense against infection and diseases and maintenance of skin barrier function (Schmid-Wendtner *et al.,* 2006). Age plays a major role in producing the action of the drug on the skin because of difference in PH. The moisture content of skin at different parts of the body seems to influential in determining the ph. Parts like axilla, submammary folds and finger webs seems to have slightly higher ph than the other body parts. Race and ethnicity also have reported of changes in ph of the skin; investigatory studies have shown that people with black skin have slightly acidic than people with white skin (Dikstein *et al.,* 1989). External factor like skin cleansing is a noticeable factor for raising the skin ph. Alkaline soaps, synthetic detergents or even tap water raise the ph of the skin even its for a shorter period, the effect only remains for the upper layers of the stratum corneum. Applying drugs and cosmetics on the surface of the skin can increase the pH and cause skin irritation even though the skin has a buffering capacity. Most of the reported cases of skin irritation showed that used cosmetic or drug is having an alkaline solution of pH above 7. The ADRs was found to be increased transition temperature and swelling of stratum corneum lipids and disruption of skin barrier function characterised by increased transepidermal water loss (Barel *et al.,* 2001)

Parameters affecting drug absorption

For effective drug therapy via skin, understanding the core parameters that enhance the drug absorption is vital. In order to produce a local effect in the body, the drug is formulated in the form of ointments, creams which are semisolid. The adhesive patches provide systemic action. In both cases, the drug has to cross through stratum corneum to produce a therapeutic effect (Williams, 2003)

The typical transdermal delivery system is a passive process. Fick's law governs this process.That is, the rate of absorption or flux of any substance across a barrier is proportional to its concentration difference across that barrier (Michaels *et al.,* 1975). Drug potency and therapeutic effectiveness mainly depend on the vehicle and Influence the rate of absorption. Extensive pharmaceutical research has shown that the same concentration of particular drug in different formulations gives contrasting action. Drug concentration of the soluble drug makes the driving force for percutaneous absorption. Higher drug concentration in a formulation can be advantageous for certain drug which has to be worn for a long period, but not for all. In order to maintain the equilibrium of constant drug concentration and delivery, non-dissolved drug dissolves eventually and absorb by the skin (Stoughton,1992).

By topical application, only a small partition of the drug is entered into the stratum corneum. Remaining drugs get lose through sweating, exfoliation, wash-off, rub-off, photochemical degradation, absorption onto clothing (Jayakumar, 2016). In case of patches for a long duration, half of the amount of drug still present in the patch may result in safety hazards. Hydration of the occluded skin can expand the reservoir volume of drug in the stratum corneum and increase its absorption up to 5-10 folds. Burning, stinging and contact dermatitis may occur when it is applied to fissured or eroded skin. Permeability and absorption may vary from site to site. The rate of absorption greatly depends on the depth of stratum corneum, the number of the sebaceous gland and hydration status (Bronaugh *et al.,* 2005).

Vesicular drug delivery system

Vesicular drug delivery system is newer and versatile controlled drug delivery approach to meet the demands of the body with the use of new and pre-existing drugs. It is introduced in order to reduce toxicity, side effects, maintain the therapeutical value of the drug, increases the bioavailability and reduce the cost of the drug (Cartolla *et al.,* 2016). Both lipophilic and hydrophilic drugs can be used to produce desired effects. It also improves effectiveness by drug transport through the various biological system (Saurabh *et al.,* 2012). They function as a sustained drug delivery system by delay the elimination of the metabolised drug. It acts as a promising system where it overcomes drug instability, insolubility and rapid degradation (Aswathy *et al.,* 2013). Carriers are important for the successful transport of drug. They are drug vectors which carry, store and deliver it to the desired target. They can interact with a biological system and are engineered in such a way that it releases the drug slowly (Vyas *et al.,* 2002). The carrier should be able to cross biological barriers and tumour vasculature in case of tumour chemotherapy. Once internalised by the body, it should be non-toxic, non- immunogenic, biodegradable, sterile for parenteral use, easy and inexpensive to prepare. Different types of vesicular systems include lipoidal and non-lipoidal bio carriers (Fathima *et al.,* 2016)

Lipoidal biocarriers

- Liposomes
- Emulsomes
- Enzymosomes
- Ethosomes
- Sphingosomes
- Transferosomes
- Pharmacosomes
- Virosomes (Sunil *et al.,* 2013)

Non- lipoidal bio carriers

- Niosomes
- Bilosomes
- Aquasomes.

Liposomes

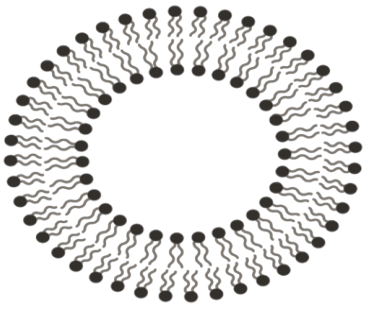

Figure 2: Structure of liposome

Liposomes are concentric vesicles which made up of a phospholipid bilayer and encloses aqueous volume inside it (figure 2). These phospholipids are having a hydrophilic head (water-attracting) and a hydrophobic tail (water repellent) made of long-chain hydrocarbons. A liposome is containing a drug used for the delivery of drugs for cancer, skin disease and another life-threatening disease (Pajaree *et al.,* 2018). It improves the dissolving ability of insoluble drugs, can be easily digested in the intestine and thus the drug can be released. It enhances ADME (absorption, distribution, metabolism, excretion) of the drug (Mansoori *et al.,* 2012). It also decreases the lethal effect of the encapsulated drug in the liposome and thereby improves the stability, therapeutic effect of the drug. The main therapeutic application of this include inhibition of prostaglandin and decrease intra ocular pressure (IOP) of the eye. They are also incorporated in ergosterol membrane and phosphodiesterase. The drug released from the liposome and physical stability of the system depends on membrane fluidity and which is determined by lipid composition. It consumes more time for the release of drug from the formulations. Due to its high production cost, less stability, low solubility, leakage of encapsulated drugs from liposomes, and their use is limited in certain cases and for some products(Dua *et al.,* 2012). There are different types of liposomes prepared, and thus they are classified according to their structure parameter, liposomal preparation, composition and application, speciality liposome, a conventional liposome (Perrett *et al.,* 1991).

Bicelles

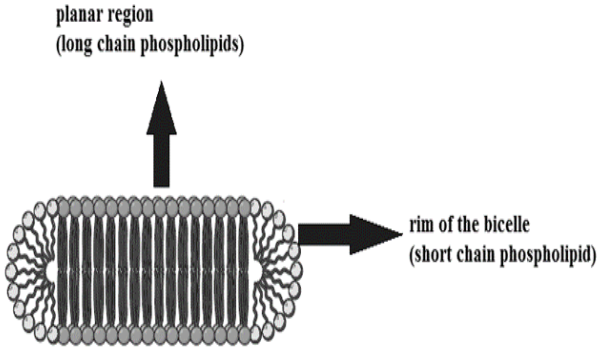

Figure 3: Structure of bicelle

Bicelles have great significance in studying membrane-associated biomolecules. It is phospholipid aggregate of long chain and detergents (figure 3). This detergent rim present in the bicelles shields hydrophobic phospholipids tail portion from the water. It is also a transitional model membrane between micelles and lipid vesicles (Gabriel *et al.,* 1984). The morphology of these structures vary with different factors, and they tend to be specific for it. Because any change in the factors can directly affect the bicelles

structure and its morphology and thereby producing different actions. The total concentration of phospholipids which is used to make the bicelle is the major and important one in deciding the morphology of the bicelles. Long chain and short chain fatty acids are used to prepare this bicelle like acyl cationic lipids, acyl cationic lipids, zwitterionic acyl lipids etc. the ratio of this taken phospholipids also determines the morphological characteristics of bicelles. The temperature at which it is prepared significantly changes its structure. Hydration is also a factor like the above ones (Whiles *et al.,* 2002).

Advantages of bicelles

- The main benefit of bicelles is that they can orient themselves in the magnetic field and can be modulated as a real molecular goniometer.
- Ability to encapsulate different molecules in their structure.
- The absence of surfactants in the composition o fbicelles.
- They can impersonate a certain type of cell membrane
- The structural resemblance with the lipid layers of stratum corneum.
- Sustain key bilayer property.
- They are effectively monodispersing, non-compartmentalized, optically transparent.
- Enhance drug penetration by modulating the skin barrier function and biophysical properties.
- Since it is having a nanostructure and has the ability to penetrate the intracellular spaces of stratum corneum, considered as a potential carrier for the dermal application (Barbosa *et al.,* 2012).

Different lipids used for bicelle preparation

- Ether Anionic Lipids
- Acyl Zwitterionic Lipids
- Acyl Anionic Lipids
- Acyl Cationic Lipids
- Ether Zwitterionic
- Ether Cationic
- Lanthanide Chelating Lipids
- Ether Anionic Lipids

Bicosomes

Bicosomes are defined as bicelles encapsulated in liposomes (figure 4 and figure 5). It acts as a permeability enhancer of the biological membrane, allow encapsulation of drugs, dyes and other pigments in dermatology, cosmetics and pharmaceutical interest (Leng *et al.,* 2003). Bicelles lose their properties and efficacy when they are in dilute solutions by altering into larger structures. Without changing the morphology, bicosome preserves and isolates bicelles from environments with high water contents (blood, mucous membrane, eye). Thus bicosome effectively reaches different tissues and produce its action. They isolate and protect these nanostructures from the dilute medium in the body. The exterior lipid membrane provides stability and efficacy to the discoidal bicellar system and serves as a perfect microenvironment. Liposomes are independent of temperature and dilution and maintain controllable size. The encapsulated bicosomes are not at all affected by the dilution system (Fasano, 1998). Recent research has shown that proteins, nucleic acid, cosmetic products, pharmaceutical and chemical applications found to be biocompatible with these vesicles. The relevance of bicosomeis that in posterior dilution they protect this nanostructure. The bicelles which are encapsulated provides a perfect microenvironment (Johnsson *et al.,* 2003). The bicosome technology provides an increase in diameter of bicelles and the number of stacking inside the liposome. Since the water content of the skin is less, increased skin permeability is seen due to the interaction of nanostructure with skin (Spellerberg *et al.,* 1995). Encapsulation of bicelles which form bicosomes are good means of introducing in biological fluids like blood, saliva; cerebrospinal fluid was high water content is seen. Apart from the application on the skin they can give intravenously (endothelium) as well as through orally (mucosa and gastric) (Cheng *et al.,* 2008).

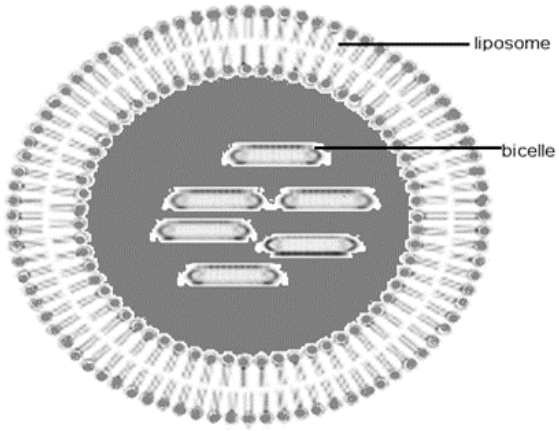

Figure 4: Structure of bicosome

Advantages

- Bicosomes combine the distinctive features of both liposomes and bicelles and emerging as a multifunctional nanostructure for modulation of biological barriers.
- Efficacy of molecules is enhanced.
- It is applied to different tissues like mucosa other than skin (Guo *et al.,* 2008).

- Easy and gentle penetration and remain in the target layer for a sustained release.
- Products applied on the skin do not penetrate other layers and effects disappears just after one wash.
- Do not penetrate aggressively into the skin and systemic toxicity is less (Rubio *et al.,* 2010).
- Exert a protective effect on collagen fibres from degradation induced by IR radiation.
- Reinforce skin lipid structure by addition of new lipids and by increasing the order in the lipid organisation.
- Treatment of the skin with Bicosome including beta-carotene inhibits the formation of free radical in the skin by UV radiation with higher efficacy than a commercial sunscreen (WashingtonC *et al.,* 1996).
- Bicosome increases the retention of drug in the tissue.
- Reduces collagen degradation due to the exposure of IR radiation (Gaede *et al.,* 2003).

Preparation of bicosome

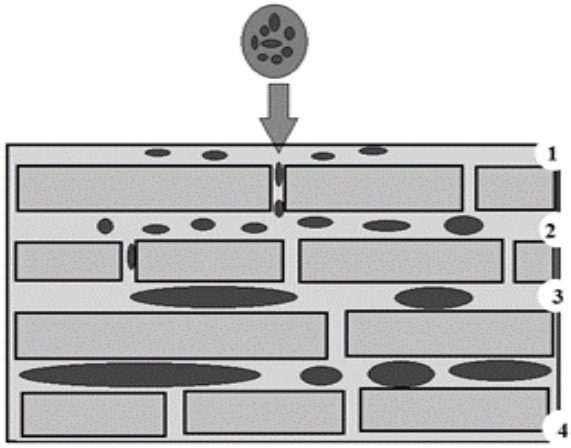

Figure 5: Structural changes of bicosome

Liposome which is having a composition of 80% of Lipoid-S and 20% cholesterol were selected. These components were mixed in chloroform. Remove the chloroform by rotary evaporation to form a lipid film. The previously prepared bicellar solution was used to hydrate the lipid film. In this step, a portion of lipids of the film is added to the disc bilayer followed by the incorporation of bicellar lipids into the outer membrane of the bicosome. Through polycarbonate membrane (800 nm) this solution was thrust out. By process of centrifugation, bicosomes are isolated and collected. The disc diameter found to depend on lipid redistribution. Increase in the diameter of the disc shows that these structures can stalk inside the vesicle (Gelen *et al.,* 2010).

Characterisation of bicosome

Dynamic light scattering: Size of smart structures is appropriate to penetrate between the narrow intercellular spaces of the outer most layer of the skin, the stratum corneum (Schmitz *et al.,* 1990).

X-Ray scattering: Bicosomeis structurally based on lipid bilayers similar to those present in the skin.

Microscopy: Great morphological versatility, these structures are able to adopt different sizes and shape depending on composition and environment (Bouwstra *et al.,* 1993).

Table 1: showing the size of the skin layers (RossMH *et al.,* 1989)

Layer of skin	Size
Epidermis	0.2 mm
Stratum spinosum	50-150 micrometre
Stratum granulosum	3 micrometre
Stratum corneum	8-15 micrometre
Viable epidermis	10 micrometre
Dermis	2 mm
Hypodermis	4-9mm

Table 2: Materials for bicosome (Estibalitz *et al.,* 2016)

Materials	Concentration (w/v%)
Long chain phospholipid (DPPC)	4.25%
Short chain phospholipid(DHPC)	0.75%
Pigment (β-carotene)	0.01%
Steroid (CHOL)	2%
PC	8%

Applications

Bicosomes are the newer, developing drug delivery systems which have startedan increased fame in different sectors of industry and research purposes. Its highly efficient ability to maintain its morphological structure in a different environment and to successfully transport the drug into the desired place its make it highly applicable in different pharmaceutical drug delivery systems. The new era of transdermal drug delivery system is opened by this bicosomes wherein they carry and protect the drug moiety from the hostile environment in the body and to produce the desired effect. It is used as an efficient drug carrier and a drug modulator of drugs that need systemic activity. Antibiotics, anti-inflammatory and vitamins are now currently incorporated in these vesicular structures for evaluating its efficiency. Bicosomes are integrated with various drugs and medications for various diseases. Skin lipids such as ceramides, cholesterol,

cholesteryl sulfate.In the bicosome platform, various drugs such as anti-histamines, anti-fungal and antibiotics etc. has been established in recent researches they are also used as a diagnostic marker for the differential diagnosis of diseases. For diagnostic purposes, compounds such as iron, fluorescent probes and magnetic resonance marker have been employed. Since its application is more effective to cosmetic skin industry can make a huge advantage out of it. Different cosmetic preparations like sunscreens which protect the skin from harmful UV radiations, anti-ageing creams and lotions, body lotions for producing hydration effect on the skin can be incorporated in these structures and produce action promptly and effectively. They have the ability to remain in the skin layer for a longer period, so the number of application of the drug is reduced. It's also widely used in cosmetic products and the actives are Caffeine, vitamins (A, E or C), antioxidants. The drugs (antioxidants) which are active in lipid environment such as beta- carotene, trap the free radicals and oxygen radicals and thus protects the collagen fibres of the skin. Not the drugs but also the lipids which bicosome made of will absorbs the infrared radiation and thus protect the skin from further damage. Apart from the dermatological field their application also extends to textile industries. They increase the absorption, penetration and distribution of dyes into the textile fibres. Manufacturing of smart fibres is also a new developing technique using bicosome technology. Finally, the application of these vesicular particles is gaining importance day by day. The promising features of these structures are making the pharmaceutical industry open to newer options for drug delivery and increasing its efficacy (Shah *et al.,* 2016).

Bicosomes interaction with skin

The application of this system for skin purposes is yet to be explored. The outermost layer of the skin constitutes only 10-20% of the water content. These low amount of water helps to improve the stability of discoidal bicelles and interaction of these structures with skin. This interaction promotes the effectiveness of the barrier. Bicosome exerts a protective effect on collagen fibres from degradation induced by IR radiation. Bicosomes penetrate and remains in the skin. Bicosome reinforces the skin lipid structure at two levels. New lamellar structures are formed inside the tissue, and other are detected on the skin surface. Bicosome reinforces the skin lipid structure by addition of new lipids and by increasing the order in the lipid organisation (Lundquist *et al.,* 2006). Treatment of the skin with bicosome including beta-carotene inhibits the formation of free radical in the skin by UV radiation with higher efficacy than a commercial sunscreen. Bicosome increases the retention of drug in the tissue.

- Unique structures of bicosomes are small enough to pass through stratum corneum of the skin without any damage to tissues.
- Once they are inside the cell, structural transformation occurs and increase its size.
- These structural transformation helps to hold the bicosomes in between the skin layers and allows slow drug delivery of the drug included in the bicosome
- Higher efficacy and long-lasting effects are maintained until this layer is lost by the desquamation process (figure 6) (Krutmann *et al.,* 2012).

CONCLUSION

A number of skin diseases are chronic and need proper treatment. Newly developed bicosome technology is a multifaceted platform for the reduction of skin disorders. The main application of this system in the dermatological field is that its ability to restore the skin barrier function and to spot the specific skin layer. The mechanism of action is that its run the active substance directly into the specific skin layer and exerts the action. The efficacy of the molecules is increased when they are carried in bicosomes. Our main target is the skin, but this delivery system can also be applied to other different tissues like mucosa. The way to market for Bicosome health care products is through partnering with larger pharmaceutical companies. Additionally, bicelle is able to modulate the biophysical parameters and barrier function of the skin. Given these properties, these nanostructures appear to be smart nanosystems with great potential in biomedicine and dermopharmacy.

Conflict of interest: All authors have none to declare.

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