Flexible Liposomes for topical Applications in Cosmetics

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Abstract

Liposomes are commonly used in dermal applications as protective systems for active ingredients and for their moisturising properties. They are spherical vesicles composed of phospholipids with an aqueous core. Either lipophilic or hydrophilic active ingredients can be incorporated in these vesicles. But they may also have the property to penetrate into the skin, carrying actives to the target site, where these molecules will be released. Liposomes acting as such a dermal carrier have to be small sized, unilamellar and equipped with a flexible membrane.

1. Introduction

Nowadays skin care formulations must meet high standards of efficacy - preferably visible effects – for the consumers are much more sophisticated than in the past. As a result, consumers expect and demand real performance from their products. To ensure effectiveness of the cosmetic formulation, the actives have to be transported to the target site, mostly into the epidermis.

But penetration of substances through the skin is limited by the natural barrier – the stratum corneum with its "brick and mortar" architecture. Indeed, the interstices in the horny layer, which seldom exceed more than 20 nm in width, are extremely impermeable even for small molecules. Water actually trespasses the skin at the rate of 0.4 mg cm^{-2} h^{-1}.

To overcome the skin barrier, chemical enhancers may be used which typically increase the fluidity of the lipids in the stratum corneum (sc).[1] Despite the fact that Alec Bangham published the first paper on liposomes in 1963, it was in the early 1980s that Mezei and Gulasekharam reported the effectiveness of liposomes in topical drug delivery.[2,3]

2. Liposomes

Liposomes are hollow spheres that are enclosed by one or more bilayer membranes. These bilayer membranes consist of natural components as for example phospholipids – in particular phosphatidylcholine (PC), which make these carrier systems biocompatible per se. PC is obtained either from soy beans or eggs, which differs in its composition of fatty acids. Egg-derived lipids have a higher content of saturated fatty acids (40% of 16:0 and 18:0) in comparison to soy bean (80% of 18:1 and 18:2).[4]

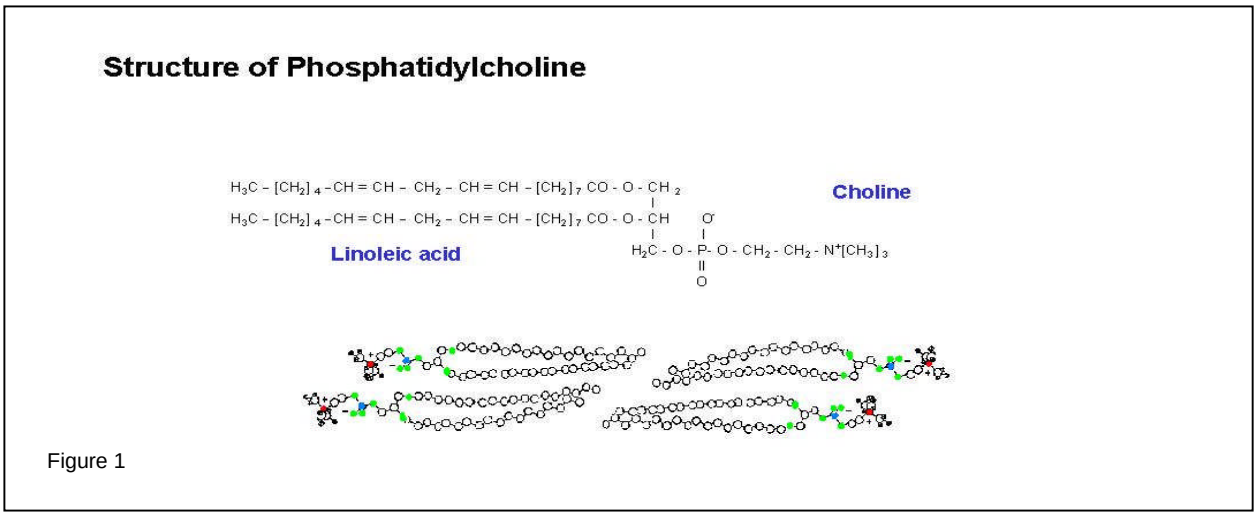

Figure 1

The amphiphilic nature of PC allows them to self-aggregate in an aqueous solution and to form their spherical structures. Liposomes can be unilamellar or multilamellar as shown in **Figure 2** and their sizes range from 50 nm to several µm depending on their method of production .[4]

They are capable of delivering either hydrophilic (in the aqueous inner core) or lipophilic substances (in the lipid bilayer).

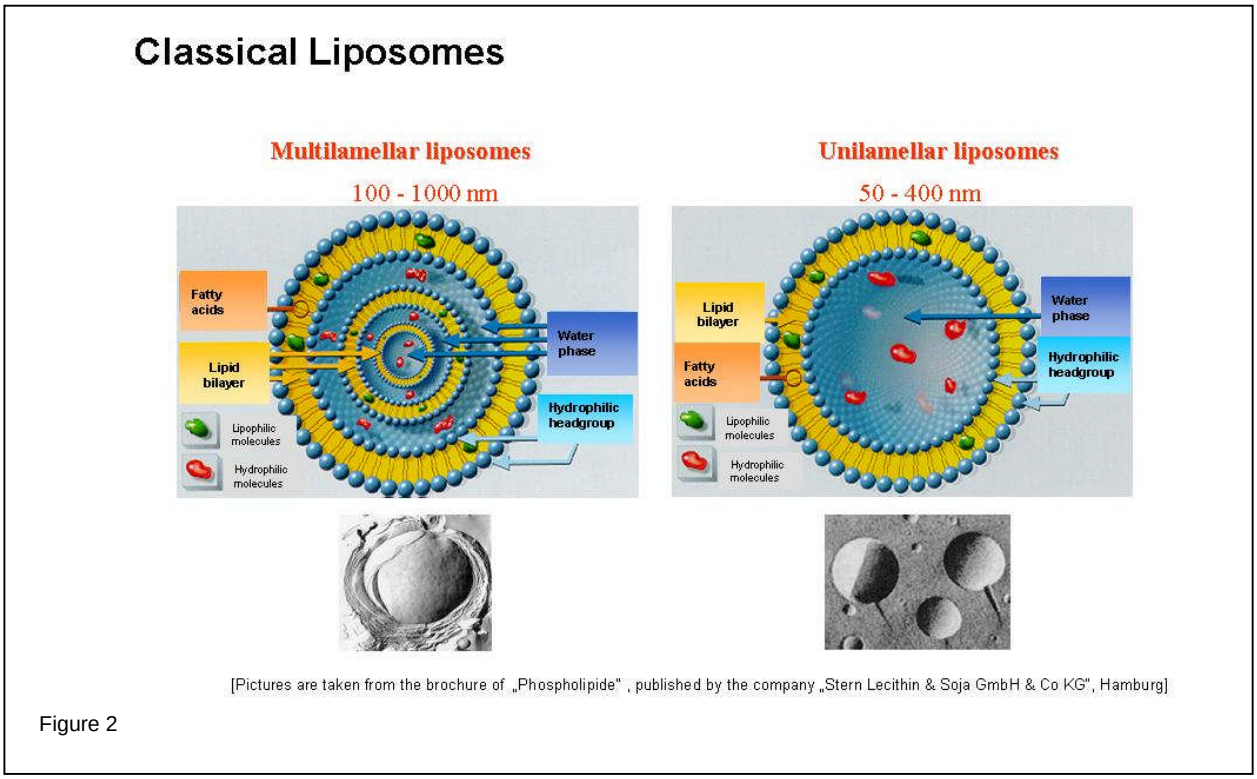

Figure 2

Increased rates of skin permeability have been found for various active ingredients, e.g. progesterone and hydrocortisone, when they were applied topically in liposomal form. Also, less frequently side effects were observed when liposomal formulations were employed.[5,6] In contrast to these observations, other investigators could not find improved skin penetration of substances when

they were applied in liposomes.[7] Also, liposomes themselves did not seem to penetrate through the sc in these early studies.[8]

The conflicting results may stem from different lipid compositions of the liposomes employed as in addition to size. The lipid composition determines the physical characteristics of the liposomes and, therefore, also the interaction of these carrier systems with the skin.[9]

In the last few years some papers highlighted important factors that influence the penetration of active ingredients encapsulated in liposomes. Liquid-state, flexible liposomes showed greater skin penetration than those in a gel-state, small-sized and unilamellar vesicles seem to result in a higher degree of skin penetration.[10-12] The application form can also influence the penetration kinetics.[13]

3. Flexible Liposomes

Flexible liposomes are small-sized unilamellar vesicles (80-250 nm) prepared of soy bean phosphatidylcholine (> 80%) having a high content of linoleic acid. They provide the skin with essential polyunsaturated fatty acids (vitamin F) which support the formation of ceramide 1 and with choline which is a part of the natural moisturising factor (NMF). In a clinical study it was proven that these liposomes have cosmetic properties like wrinkle reduction and an increase in skin smoothness and furthermore show pharmaceutical effects like decreasing of efflorescence in the acne treatment.[14,15]

3.1 Penetration

The demands to bring active ingredients into the deeper skin layer and to get a site-specific targeting of these molecules with visible cosmetic effects become more and more relevant.

In an ex-vivo study on human skin biopsies, fluorescence markers- liposomally encapsulated or in a free form - were tested on their ability to penetrate into the skin. The penetration profile was visualised by confocal laser scanning microscopy (CLSM).

The hydrophilic fluorescent dye carboxyfluorescein (CF) and the lipophilic dye 1,1-diocytadecyl-3,3,3,3,-tetramethylindocarbo-cyanine perchlorate (DiI) were encapsulated together in liposomes made of soybean lecithin (PC > 80%) by homogenization and subsequent extrusion through a 0.1 µm microporous filter .

Human abdomen skin was obtained after cosmetic surgery. After removal of subcutaneous fat, the skin was placed in Franz-type diffusion cells. The liposome preparation and the CF-DiI ethanolic solution (control) were non-occlusively applied on the epidermal side of the skin. Usually 50 µl of test preparation were pipetted onto a circular area of 3.5 cm diameter. Three hours after application the skin surface was rinsed several times with buffer and blotted dry. After this, skin cylinders were

punched, cryo-fixed with CO_2 and cut in 10 µm thick pieces. After air drying the specimens were examined by CLSM (BioRad MRC 1024).

Ethanol itself is a moderate skin penetration enhancer for many substances.[16] However, as can be seen from the **Figure 3**, an ethanolic solution of the fluorescence dyes is not able to penetrate noticeably through the stratum corneum during the observed period of time. In contrast, the dyes encapsulated in the flexible liposomes appear to penetrate through the stratum corneum and enter the deeper layers of the epidermis. Furthermore the visible fluorescence in the hair follicles indicates the transportation of molecules through the hair shaft as far as the hair roots **(Figure 4)**.

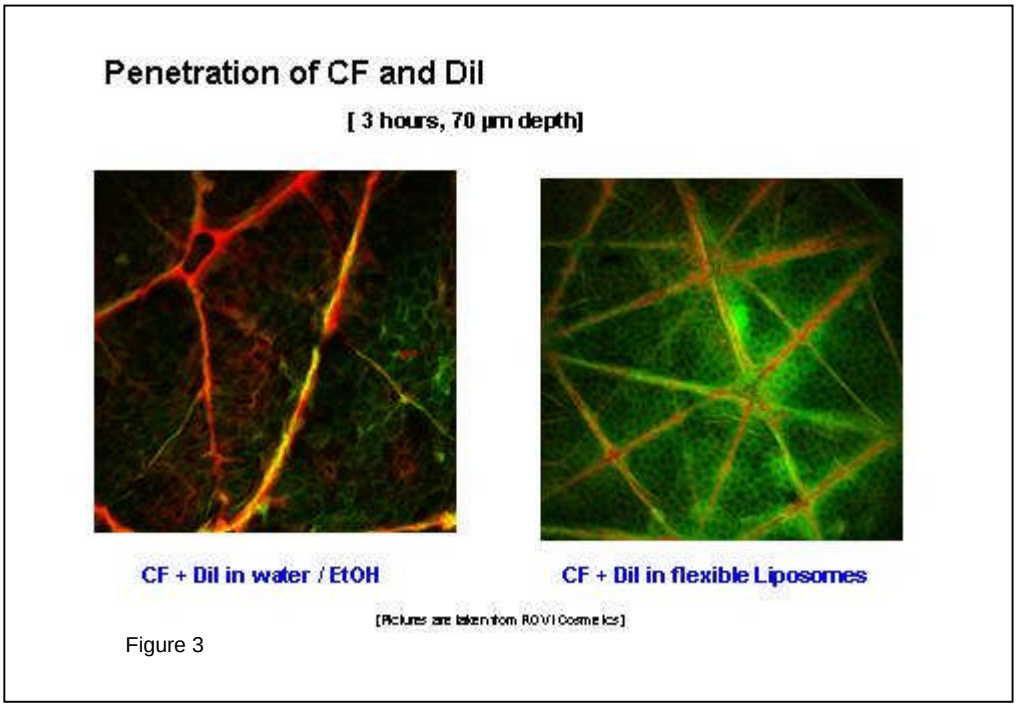

Figure 3

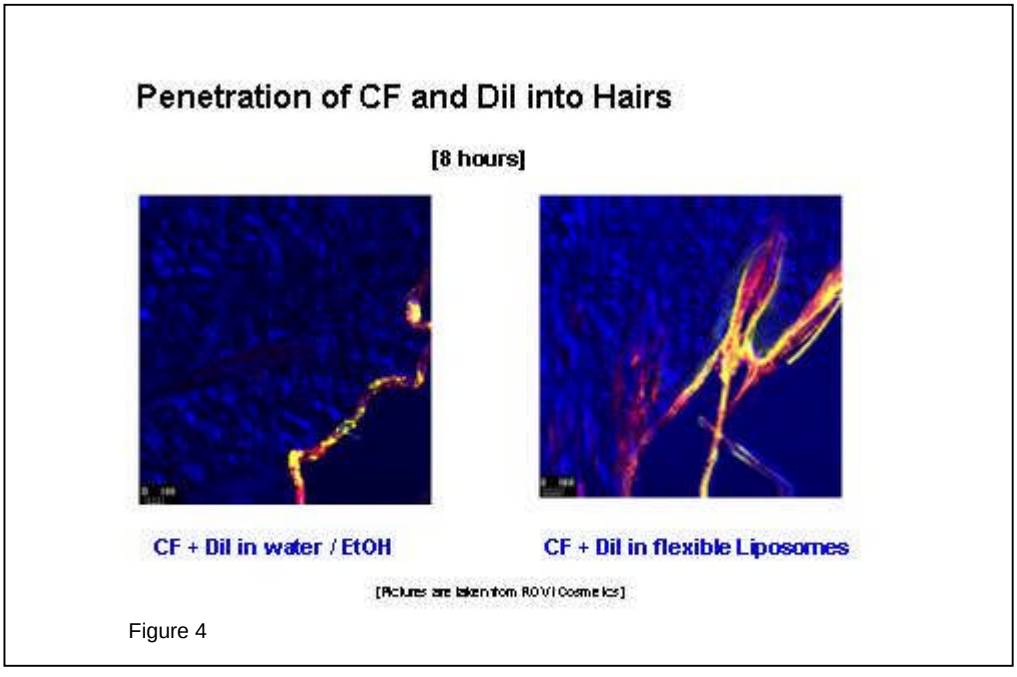

Figure 4

From these and earlier findings, it is fair to assume that at least a part of the liposomes will cross the stratum corneum intact - a tentative model of the penetration behaviour of these liposomes is depicted in **Figure 5**. Previous studies have shown that the ratio of lipophilic to hydrophilic ingredients, both encapsulated in liposomes, did not change during the passage through the stratum corneum. If the liposome structure would have be destroyed during their transport through the stratum corneum, a strong increase of the hydrophilic component would have been observed due to the release.

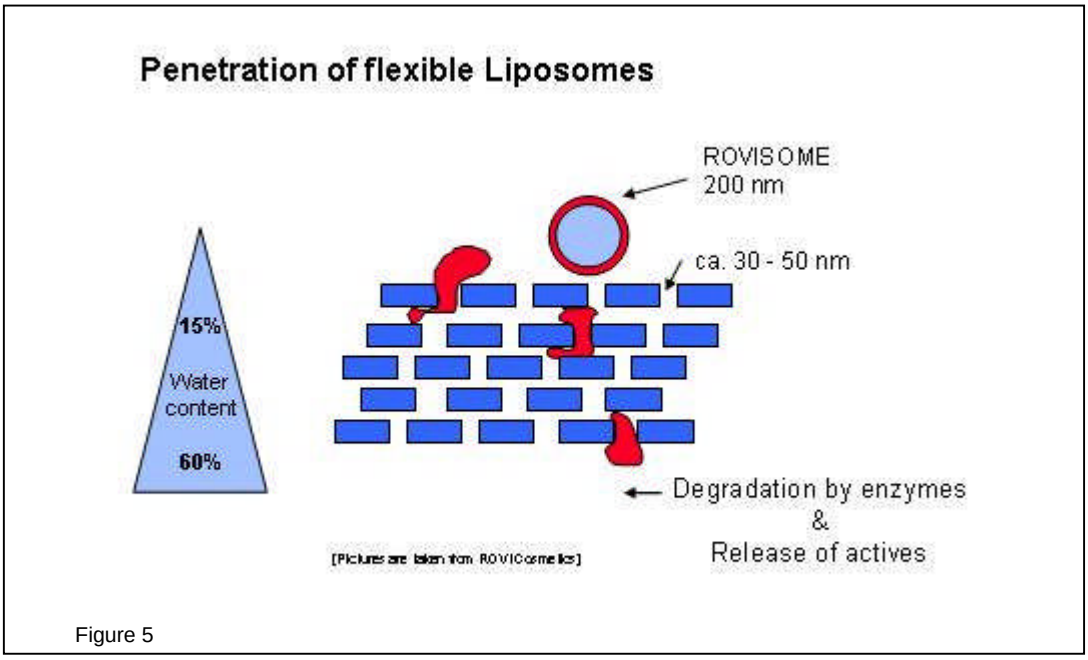

Figure 5

The driving force for the movement of the flexible liposomes (and their payload) is presumably generated by the hydration gradient across the skin, which varies from 15 – 20% water content in the sc up to 70 % in the stratum granulosum.[13] When the flexible liposomes are applied onto the skin and allowed to dry, the vesicles are attracted by the moisture in the epidermis and due to their flexibility they penetrate the skin.

Vitamin E acetate encapsulated in flexible liposomes which were incorporated in a cosmetic o/w cream (lamellar) also featured a higher penetration capability of the active into the deeper skin layers.[17]

3.2 Benefits of Liposomal Encapsulation of Actives

More recently, the exclusive cosmetic raw material coenzyme Q_{10} (ubiquinone) was established to act as a potent anti-oxidant. Coenzyme Q10 plays a key role in the intracellular electron transport. It is one part of the redox system within the respiratory chain. When incorporated in a flexible liposome, the lipophilic coenzyme Q_{10} is embedded in the fluid lipid bilayer membrane just like it is

realized in natural cell membranes in the skin. This particular natural surrounding of the molecule provides the full scope of functionality and anti-oxidative property.

A new innovative electron spin resonance (ESR) spectroscopy method was introduced to the cosmetic market, which enables a benchmarked evaluation of the anti-oxidative properties (AOP) of formulations ex vivo.[18]

The penetration and activity of antioxidants can be detected by means of ESR spectroscopy on skin biopsies. For the measurement the skin biopsy is labeled with test radicals that are allowed to diffuse into the skin (epidermis) from the dermal side, but not into the stratum corneum. The cosmetic actives and/or the cosmetic formulations are applied onto the skin`s surface. If the active passes the stratum corneum and penetrates into the epidermis, it will react with the test radicals and therefore reduce their number. Accordingly, the ESR signal intensity diminishes and the penetration and penetration kinetics, respectively, can be followed. A measurement of an untreated skin biopsy accounts for the intrinsic enzymatic and non-enzymatic radical protection mechanism and has to be included in the study.

The study reveals the significant and rapid enhancement of the intrinsic protection by liposomal Q_{10} in contrast to the pure ethanolic Q10 solution as illustrated in **Figure 6**. The anti-oxidative potential becomes immediately relevant and 20 minutes after application the enhancement makes already 27%.

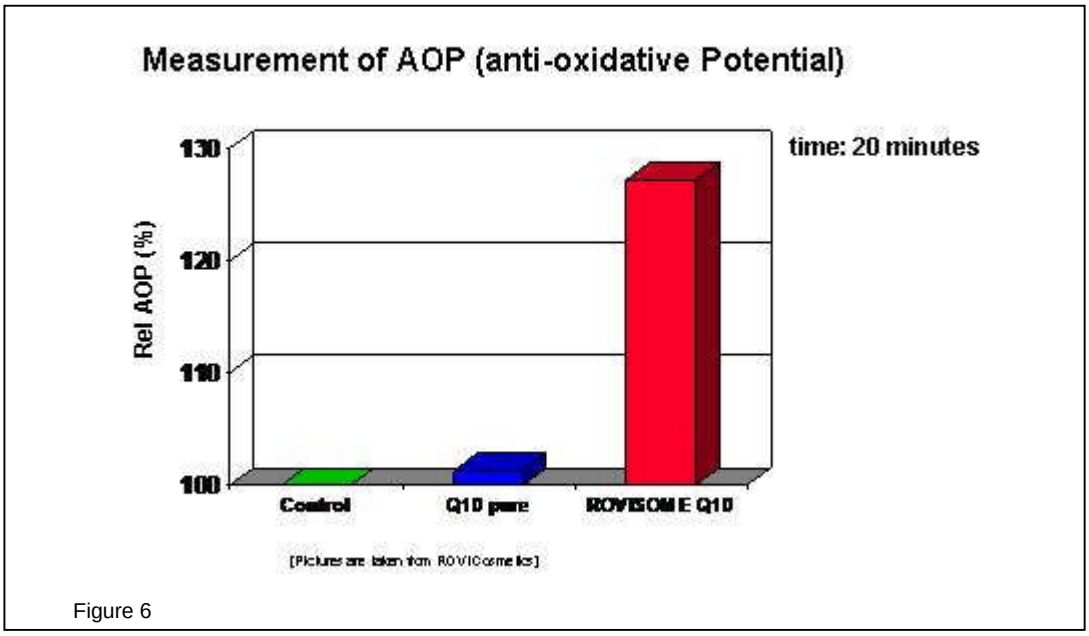

Figure 6

Sensitive ingredients (lipophilic vitamins like retinol) can be incorporated in the flexible vesicle membrane where they are protected from the outside (light, temperature, chemical degradation). This results in an improvement in the stability of the vitamin over a long time period. [19, 20]

Furthermore, the encapsulation of actives into the liposomes may avoid irritation that is caused by certain actives. Alpha hydroxy acids were introduced into cosmetics for peeling purposes in the early 1970s, due to the keratolytic and moisturising properties they possess. Generally, the solutions containing free acids or their salts were deemed intolerable by the test subjects. Of special note are the formulations containing the liposome-encapsulated AHA salts: These were judged by the subjects to be pleasant and well tolerated, causing no dermatological changes to the skin.[14]

3.3 Stability of Flexible Liposomes

Due to the high amount of unsaturated fatty acid in the liposome membrane, a fast oxidative degradation can be expected. Therefore the stability of flexible liposomes against oxidation was tested by measuring the rancidity and compared with soy bean oil which contributes a similar distribution profile of fatty acids **(Figure 7)**. The formation of the lipid bilayer significantly protects the unsaturated fatty acids against lipid per-oxidation. Therefore, this kind of liposomes can also be used as stabilized linoleic acid, which is also able to penetrate into the skin and to provoke cosmetic or pharmaceutical effects. [14,15]

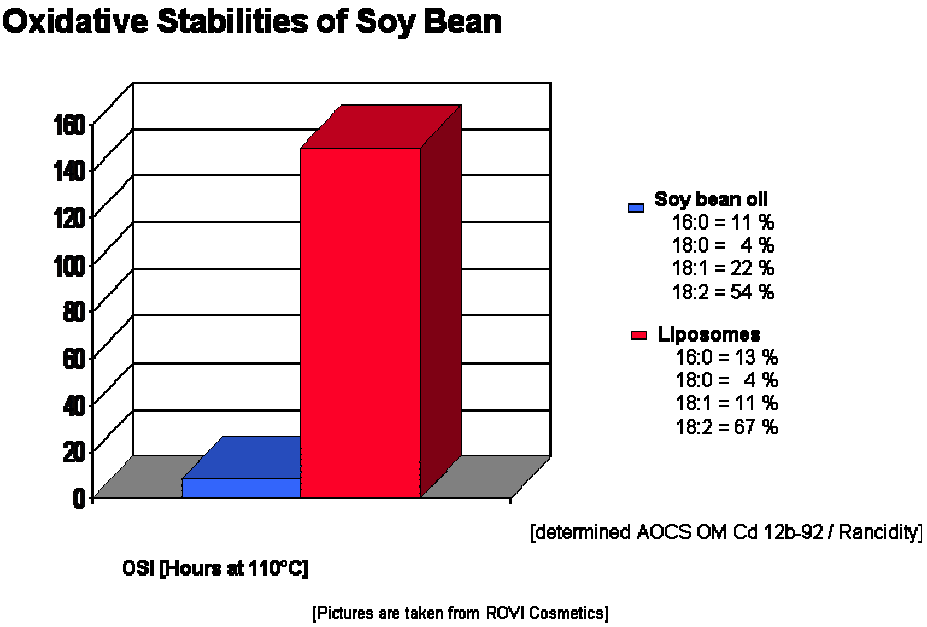

Figure 7

The first cosmetic formulation containing liposomes was introduced to the market 1987 by Dior (Capture). Since this time various liposomal formulations - including sprays, gels, lotions, emulsions, creams as well as shampoos - entered the market but without any evidence that the liposomes are still stable in these formulations. It is well known, that emulsifiers have the ability to solubilize the liposomal membrane und to release any encapsulated active ingredients.[21]

Therefore the morphological integrity of the vesicles is not any longer warranted, and the benefits of the liposomal encapsulation is affected or even nullified.

A new method to quantitatively determine the integrity and intactness of liposomes in final cosmetic or pharmaceutical formulations was recently introduced. This method is based on the previously mentioned ESR.[22] First of all, the liposomes were labeled with an ESR-active probe (chemically similar to PC) and afterwards ESR spectra were taken in different formulations as seen in **Figure 8**. By computer simulation the value of stability or degradation can be calculated.

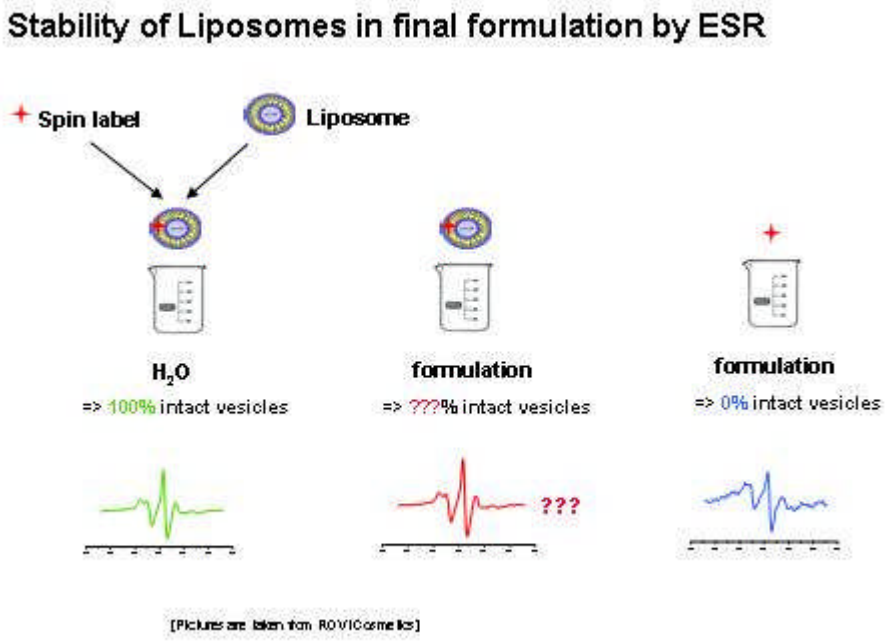

Figure 8

An excellent stability could be detected in watery systems like sprays but also in gels; in o/w emulsions or creams the stability varied between 50 and 100% (over a time period of eight weeks) depending on the emulsifiers used. Less stability (< 50%) was found in a w/o formulation and a total break down could be observed in shampoos (high content of detergents).

4. Ethosomes - Transfersomes

Recent approaches in modeling transdermal drug delivery through the skin are the design of two ultraflexible vesicular carriers – Ethosomes and Transfersomes.

Ethosomes are composed of high amounts of ethanol (45% v/v) and a low lecithin (2% w/v) concentration, which provide an Ethosome suspension with mean size of approximately 100 nm. The Ethosome suspension showed very good skin tolerability in human volunteers, also when applied for a long period (48 hours). The enhanced delivery of actives using Ethosomes over

liposomes can be ascribed to an interaction between Ethosomes and skin lipids. A possible mechanism for this interaction has been proposed. It is thought that the first part of the mechanism is due to the "ethanol effect", whereby intercalation of the ethanol into intercellular lipids enhances lipid fluidity and decreases the density of the lipid multilayer. The ethanol effect is followed by the "Ethosome effect", which includes interlipid penetration and permeation by the opening of new pathways due to the malleability and fusion of Ethosomes with skin lipids, resulting in the release of the drug in deep layers of the skin. Calorimetry and fluorescence measurements suggested that the vesicular bilayers are flexible, having a relatively low phase transition temperature (T_m) and fluorescence anisotropy compared with liposomes obtained in the absence of ethanol.[23]

Because of their unique structure, Ethosomes are able to encapsulate and deliver through the skin **(Figure 9)** highly lipophilic molecules such as cannabinoids and testosterone, as well as cationic drugs such as propranolol and trihexyphenidil. Results obtained in a double-blind two-armed randomized clinical study showed that treatment with the ethosomal acyclovir formulation significantly improved all the evaluated parameters.[24]

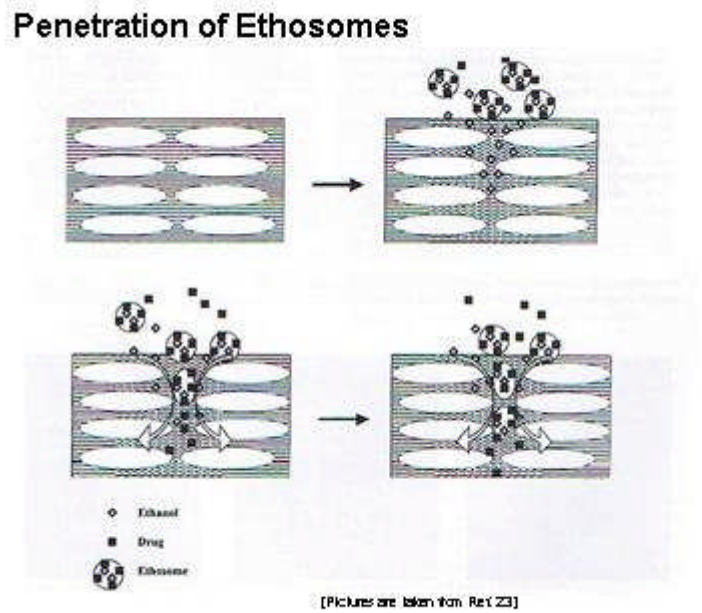

Figure 9

Due to the high concentrations of alcohol, applied with the ethosomal drug delivery system, skin irritations might be induced by solubilising the lipids of the stratum corneum and an increase in the transepidermal water loss (TEWL) might also occur.

Transfersomes are elastic, very deformable vesicles which consist of PC in combination with an edge active surfactant like sodium cholate.[13,25] This component softens the membrane of the vesicle and makes the bilayer much more flexible. Therefore Transfersomes consequently change their

shape easily by adjusting locally to ambient stress. When a suspension of Transfersome vesicles is applied non-occlusively on the surface of the skin, the water evaporates from the skin surface and the vesicles start to dry out. Due to the strong hydrophilicity, the vesicles are attracted to the areas of higher water content in the narrow gaps between adjoining cells in the skin. The phenomenon, together with the vesicle's extreme ability to deform, enables the Transfersome to temporarily open the pores through which water normally evaporates between the cells. Such newly activated inter-cellular passages can accommodate sufficiently deformable vesicles maintaining their integrity but changing their shape to fit the channel. Along these said pathways in the horny layer, Transfersomes reach regions of high water content in the deeper skin layers **(Figure 10**).

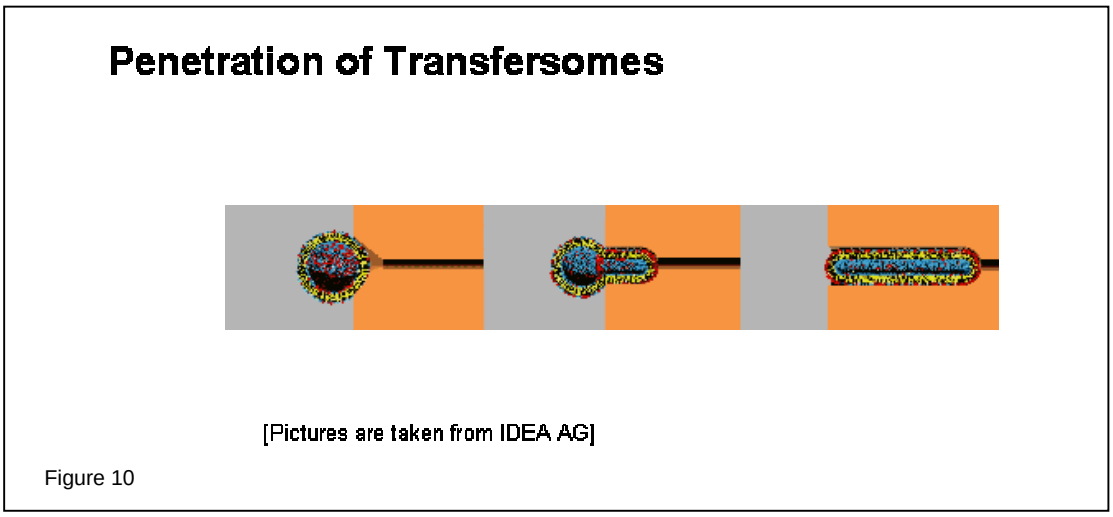

Figure 10

Being too large to enter the blood vessels locally, Transfersomes bypass the cutaneous capillary bed and reach the subcutaneous tissue. Ultimately, the vesicles may arrive into the systemic blood circulation via the fenestrated lymphatic system, which has openings (fenestrations) of sufficient width. For example, Transfersome-associated insulin is carried across the skin with an efficacy of >50% and leads to a systemic hypoglycaemia of approx 30% of that induced by subcutaneous insulin injections in humans.[26] In contrast to conventional flexible liposomes, Transfersomes are claimed to act transdermally and to carry even huge molecules (enzymes) into the blood stream.
But due to the high content of added surfactants, these vesicles are very fragile in cosmetic formulations and are not as stable as pure flexible PC liposomes.

5. Conclusions

Vesicles which really have the ability to transport active substances into the deeper skin layers or even to act as transdermal and systemic drug delivery system are only coming from the “family“ of fluid lipid vesicles like liposomes or niosomes.[9,27] Both are made from amphiphilic components –

liposomes from phospholipids and niosomes from non-ionic surfactants. The main assumption is the size and the fluidity of the membrane at room temperature which ensure the flexible deformability of the vesicles during their pathway through the stratum corneum. Liposomes prefer membrane structures that are identical to their own composition; PC is the main component of all viable cells and save in application. But liposomes hamper in their pH-dependence, their stability is best between 6.3 and 6.8.[28] Repeated applications of niosomes might lead to irritations due to the accumulation of detergents in the skin. Therefore, flexible liposomes are the only choice to carry active molecules into the deeper skin layers (biological syringe) by stabilizing the actives and even acting as pharmaceutical or cosmetic ingredient due to its chemical nature.

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