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Analysing a new generation of liposomes

ABSTRACT

When liposomes about 25 years ago first came on the market, they were initially an innovative 'magic ball' and a new argument for marketing. Later it was shown that they also worked cosmetically as an effective moisturising factor (Capture by Christian Dior).

The final breakthrough for liposomes in cosmetic and pharmaceutical applications came from the evidence that specially designed vesicles – flexible vesicles – are able to transport actives into the skin and increase their bioavailability.

This article describes the newly-introduced Sophi-Hydro-Tops, which, strictly speaking, are not liposomes – liposomes are defined as nanovesicles made of phospholipids. These new, innovative vesicles are based on membrane-forming lipids from the sunflower. The absence of phospholipids has the advantage that the discussion of soy lecithin (genetic engineering, and grubbing in the subtropics) is avoided and involvement in the growing market of 'green cosmetics' is possible.

In its cosmetic or pharmaceutical applications, the Hydro-Tops are characterised by equal or even better properties than those of the flexible liposomes in terms of penetration, encapsulation efficacy and stability.

Nowadays, skin care formulations must meet high standards of efficacy – preferably visible effects – for 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 cosmetic formulations, the actives have to be transported to the target site, mostly into the epidermis.

However, one of the major disadvantages in percutaneous drug delivery is the low penetration rate of components through the skin.

Nanovesicles are a type of drug carrier, which can protect incorporated components from degradation while enhancing their cosmetic effects by carrying them into the deeper skin layers.[1] Compared with other drug delivery systems, nanovesicles encapsulate a small volume of aqueous solution inside a membrane and dissolved hydrophilic solutes cannot readily pass through the lipids. Hydrophobic chemicals can be incorporated into the membrane, and in this way nanovesicles can carry both hydrophilic molecules and lipophilic molecules. Nanovesicles from an aqueous suspension can cross the skin barrier only through hydrophilic pathways.[2]

In cosmetics, liposomes are predominantly formed by phospholipids of natural, soy bean-based phosphatidylcholines. Although there may be some variety both in the chain length of the fatty acids and in the degree of unsaturation, the lipophilicity of the alkyl chain remains relatively unchanged.

'Deformable' or 'flexible' liposomes were introduced 20 years ago, which are capable dermal delivery systems for low as well as high molecular weight drugs. Materials commonly used for the preparation of these vesicles are phosphatidylcholines with unsaturated fatty acids, surfactants for providing flexibility, alcohol as a solvent and buffering agent as a hydrating medium. Because of their good penetration power and flexibility, deformable liposomes are used for an effective delivery of a wide range of cosmetic and pharmaceutical actives. Deformable liposomes have reported dermal delivery of actives, when applied non-occlusively. The main advantage of these deformable vesicular systems is the elasticity of the bilayer, given by the linoleic acid found in soy bean phosphatidylcholines (normal flexible liposomes).[3] Ultra-deformable vesicles are phospholipid vesicles with the addition of surfactants in the case of transfersomes (pegylated surfactants) or with the addition of terpenes (e.g. limonene) in the case of invasomes or ethanol in the case of ethosomes (fluidisation of SC lipids).[4–6] The elasticity allows all of them to squeeze through channels in the *stratum corneum* that are less than one tenth the diameter of the vesicles.

At present the idea for the development of the new Hydro-Tops (now referred to as 'the new sunflower lipids') was to get a nanovesicle with the same or even better penetration profile than conventional flexible liposomes but based of non GMO derived membrane forming material. The soy plants, and thus the soy lipids, nevertheless still carry the stigma of 'genetic modification' and cultivation that has a negative impact on nature. Rain forest and savannah in South America must vanish as the Neue Zürcher Zeitung newspaper recently wrote.[7] Therefore the new sunflower lipids were designed to consist of a membrane-forming, edible anionic emulsifier being structurally similar to phosphatidylcholines but derived from naturally growing sunflower. For stabilisation reasons, a further plant derived emollient is added.

Experiments

Materials

Unsaturated soybean phosphatidylcholines (PL 80) dissolved in ethanol (NAT 8539 = PC : ethanol = 75:25 w/w) was a gift from Phospholipid (Cologne, Germany). Imwitor 375 was a gift from Sasol (Witten, Germany) and Crodamol OE was offered by Croda (Nettetal, Germany). 5 (6) Carboxyfluorescein (CF) was purchased

Table 1: Size of nanovesicles and polydispersity index (PDI).

	Empty vesicles		CF (376 Da)		FITC Dext. (250,000 Da)	
	Lipos	Tops	Lipos	Tops	Lipos	Tops
Size (nm)	103	73	82	60	242	65
PDI	0.34	0.08	0.26	0.13	0.34	0.11

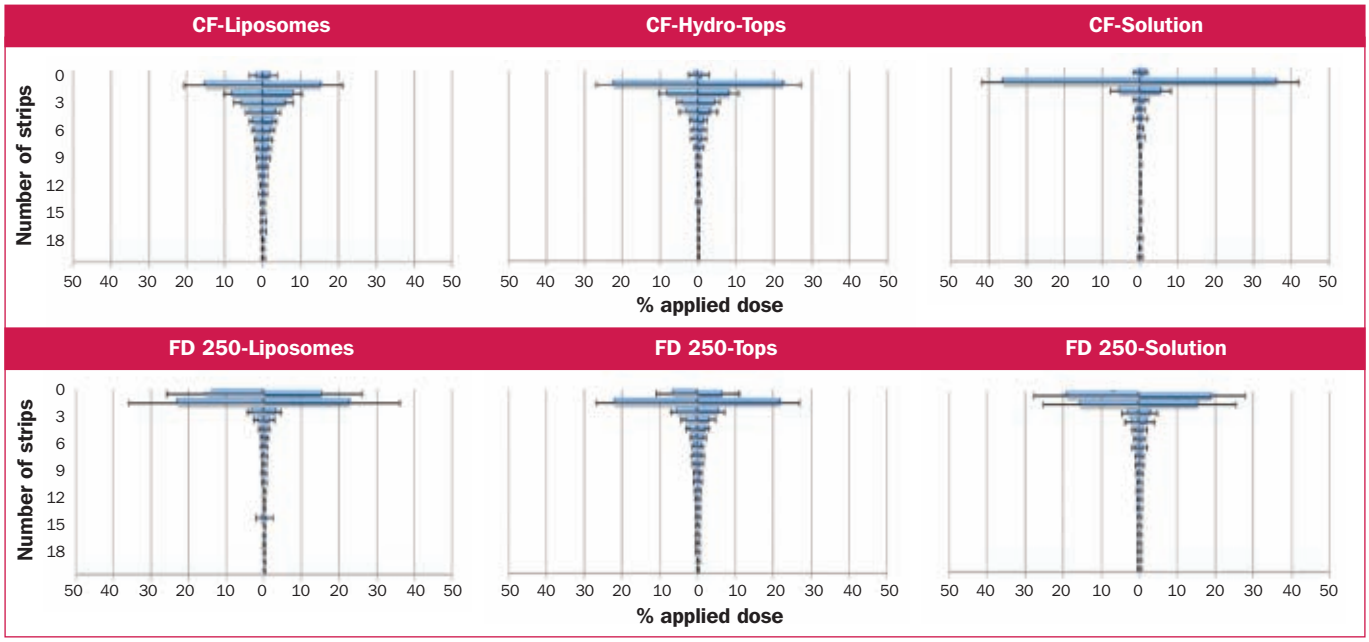

Figure 1: *Penetration of CF and FITC dextran into the skin two hours after application.*

from Fluka Biochemika (Steinheim, Germany); Fluorescein isothiocyanate-dextran FD200 (MW = 250.000 Da) was purchased from Sigma Aldrich. All other chemicals were of analytical grade.

Vesicles preparation

Vesicles were prepared by the following method: 740 mg of 2.5 mM CF or FITC-dextran solution was dissolved in ethanolic lipid solution (160 mg ethanol + 100 mg lipid). Hereby liposomes were prepared from soy bean phosphatidylcholines and the new sunflower lipids from Imwitor 375 and Crodamol OE. The mixture was vortexed for 5 minutes and afterwards sonicated for 5 minutes in order to obtain a clear vesicle dispersion. Ethanolic solutions with the same amount of fluorescence dye were used as control.

Skin penetration studies

Skin preparation: Permeation studies give an important insight into the drug behaviour *in vivo*, since the amount of drug permeated dictates the amount of drug available for cosmetic or pharmaceutical effects. Excised human skin from patients who had undergone abdominal plastic surgery was used. For the permeation studies epidermis and full skin were used. Epidermal membranes were prepared by heat separation technique. Whole skin was immersed in water at 60˚C for 2 minutes, followed by careful removal of the epidermis.

Ex vivo skin penetration study: The skin penetration profile of CF and FITC-dextran after 2 hours' application was examined by tape stripping. For these penetration studies the Saarbrücken penetration model was used.[8] Liposomes and the new sunflower lipids are non-occlusively applied onto the surface of skin (1 mg lipid/cm^2) – the lipid concentration which corresponds with the maximum uptake of phospholipids by the *stratum corneum* (SC).[9] For the purpose of removing the SC by tape stripping, 20 pieces of adhesive tape (Kristall Klar Tesa, Beiersdorf AG, Hamburg, Germany) were used. The fluorescence dyes were extracted from the adhesive tapes by adding 2 mL of a mixture of ethanol and water (1:1 w/w) to each sample and shaking them over night. The amount of dye per strip was analysed quantitatively by spectrofluorometry at an excitation wavelength of 490 nm and emission wavelength at 520 nm.

Ex vivo skin permeation study: The skin permeation of CF from different formulations was determined using vertical Franz diffusion cell. The application was chosen according to the penetration studies. In predetermined time intervals samples were taken from the acceptor compartment and the cell was refilled with an equivalent amount of fresh receptor solution. The concentration of CF in the receiving solution which means the amount permeated through the skin membrane was determined by spectrofluorometry.

Ex vivo follicular penetration study: The penetration profile of CF into the hair follicles after 16 hours' application was examined by confocal laser scan microscopy. Human hairy skin was used in combination with the Saarbrücken penetration model. After incubation of 16 hours the applied formulations were removed by wiping the skin with cotton, and cross sections (20 µm thickness) were made by the help of a cryotome. The fluorescence in the hair follicles were visualised by confocal laser scan microscopy (CLSM).

Stability of vesicles in final formulations: The integrity of vesicles in final formulations were quantified by electron spin resonance spectroscopy ESR (see Gematria Test Lab, Berlin).

Table 2: Stability of lipid vesicles in an O/W formulation.

	Stability (%)		
Vesicles	t = 0	t = 24 h	t = 48 h
Liposomes	95	95	95
Emulsifier	90	60	40
Hydro-Tops	95	90	90

INCI: Water, Jojoba ester, Steareth-2, PPG-15 Stearyl Ether, Glycerin, Canola Oil, Diocytl Adipate, Steareth-21, Dicaproyl Ether, Sheabutter, Cyclomethicone, Polyacrylamide, C13/C14 Isoparrafine, Laureth-7, Xanthan Gum, Sodium Hyaluronate, Preservative + 5% Carrier System

Results and discussion

Characterisation of the nanovesicles

The sizes of the new sunflower lipids with the encapsulated FITC dextran of high molecular weight agreed with those of the empty vesicles. All preparations show a uniform homogeneous size pattern reflecting in small values the polydispersity index (PDI <0.15). In contrast, the FITC-liposomes with sizes up to 250 nm are

more inhomogeneous (PdI >0.25), hence, greater than those of the new sunflower lipds (Table 1).

Ex vivo skin penetration study

The following figures show the penetration profile of the three formulations (solution, liposomes and the new sunflower lipids) with encapsulated CF (Fig. 1a) or FITC dextran (MW = 250.000 Da) (Fig. 1b) two hours after application.

It can be easily seen, that all formulations penetrate the first layers of the *stratum corneum* within the two-hour treatment period, however, looking deeper than the 20 strips no fluorescence could be measured. The number of strips is thus sufficient to completely remove the *stratum corneum*. Strip 0 represents the amount recovered, which could be swabbed using cotton wool and wiped from the skin surface.

The profile of CF 376 shows the best penetration property with in the case of the liposomes and the lowest penetration behaviour in solution, since already about 75% of carboxyfluorescein was found in the first strip. The dextran (MW 250.000) encapsulated in the new sunflower lipids show better penetration efficacy, whereas the liposomes remain as in the case of the solution on the skin surface or penetrate only a little in deeper skin layers.

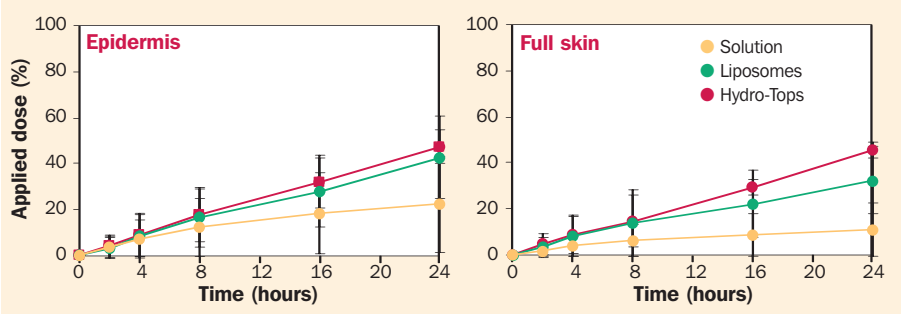

Figure 2: *Permeation of CF through epidermis and full skin by nanovesicles.*

Verma *et al*. also describe the dependence between penetration ability of flexible liposomes and sizes. Liposomes less than 120 nm in size showed a statistically enhanced CF-penetration into the skin as compared with larger ones.[10]

Ex vivo skin permeation study

The permeation of CF encapsulated in liposomes or the new sunflower lipids through the epidermis is nearly identical. But an increase of CF-permeation through full skin is observed when encapsulated in the new sunflower lipids. Using the new sunflower lipids, 46% of applied CF-dose is found in the acceptor fluid whereas 32% is found using liposomes (Fig. 2).

A good correlation can be shown between CF amount over a time period of 24 hours in epidermis and the amount determined in human full skin. Nevertheless the lowest permeation values are detected using the solution.

Ex vivo follicular penetration study

Normally pig ears are used for the *ex vivo* follicular penetration studies but in this case human skin was examined. It is very difficult to get complete parallel sections of hair follicles.[11] But from the different cross sections it was possible to detect the fluorescence within the *stratum corneum*, around the hair shaft and in the root sheath when applied by liposomes (not shown) or the new sunflower lipids (Fig. 3b). No penetration into the hair follicles was observed when the dyes were applied in the solution (Fig. 3a).

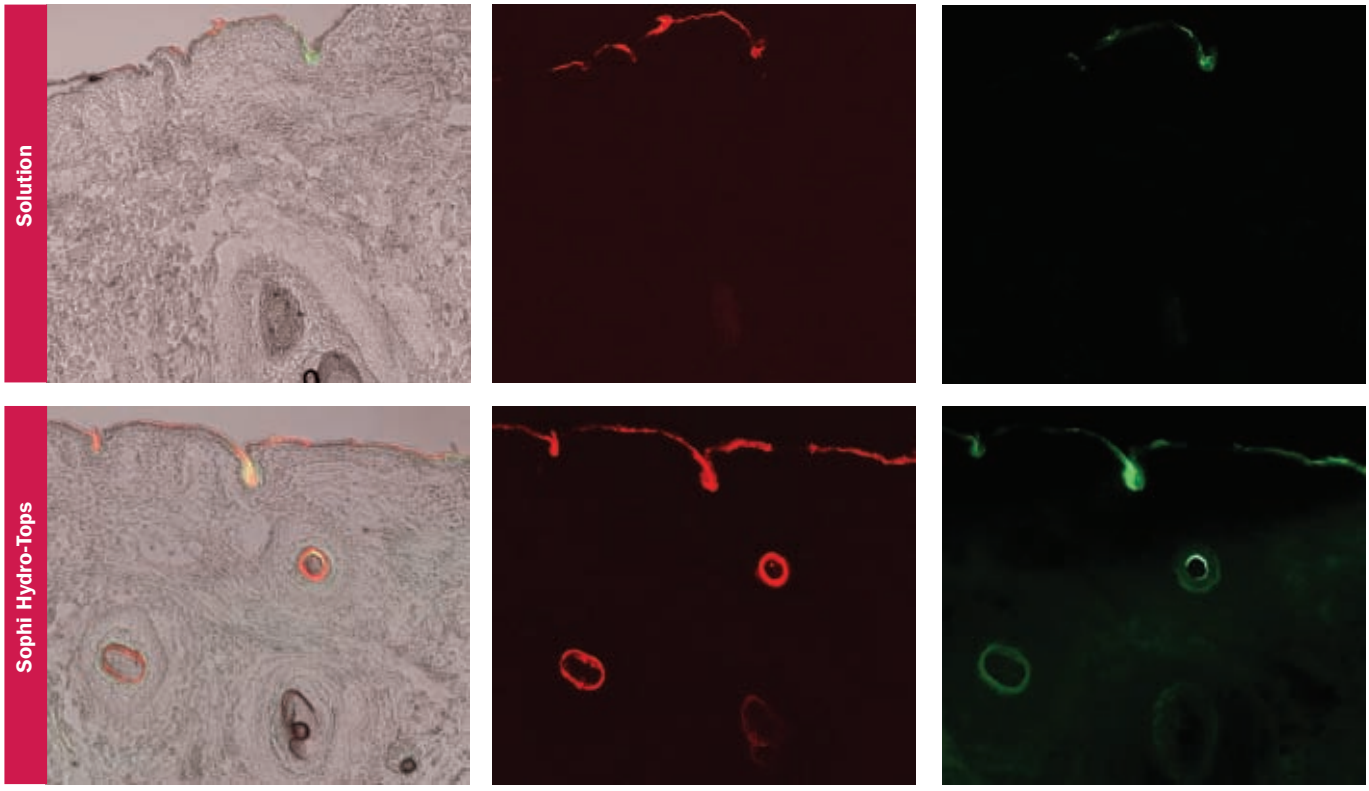

Figure 3: *Follicular penetration into human hairs* ex vivo. *(time = 16 hours)*

Stability of vesicles in final formulations

The integrity of vesicles in final formulations was quantified by ESR (see Gematria Test Lab, Berlin). An ESR-detectable spin is incorporated into the vesicle membrane. This spin shows different signals when embedded in the membrane or being set free into the formulation. Three different vesicle formulations were examined for their stability in an O/W creme: liposomes, the new sunflower lipids and vesicles only formed by the emulsifier (Imwitor 375).

Liposomes and the new sunflower lipids kept their vesicle integrity in the emulsifier containing formulation, which means no disruption of the membrane occurs. In contrast, the vesicles formed by the pure emulsifier were degraded – other structures like micelles are formed – and the encapsulated actives are set free (Table 2).

Conclusion

Nano-scaled delivery systems were first described for usage in cosmetics and pharmacy over 30 years ago; Alec Bangham published the first paper on liposomes in 1963.[12]

The newly developed Sophi-Hydro-Tops are made of a membrane-forming, edible anionic emulsifiers being structurally similar to phosphatidylcholines but derived from sunflower. For stabilisation reasons, a further plant derived emollient is incorporated.

These nanovesicles are suitable for the encapsulation of hydrophilic, lipophilic and in special of amphiphilic drugs. Compared to liposomes the Sophi-Hydro-Tops have the advantage of a higher encapsulation efficacy of amphiphilic substances and derivatives of acids like green tea[13] or ferulic acid.[14]

Regarding the penetration properties the narrow-sized, small Hydro-Tops are more efficient compared to other delivery systems. Also, a high stability of the Tops is observed in the final formulations. PC

Acknowledgements

- Many thanks to Prof. Alfred Fahr, Head of Department of Pharmaceutical Technology, Friedrich Schiller Universität, Jena for providing his help and facilities for the skin penetration/permeation studies. Also I am grateful for the assistance and support I received from Prof. Marc Schneider, Department of Pharmaceutical Nanotechnology, Universität des Saarlandes, Saarbrücken, Germany for the studies of follicular penetration visualised by CLSM.

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