Liposomes as a Carrier System for Topical Applications

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1. What are liposomes?

Liposomes are microscopic small vesicles (hollow spheres), consisting of one or more lipid bilayers that surround a watery nucleus. They are used in cosmetics as carrier systems and as stabilizers of active substances whilst regarding their moisturizing properties as well.

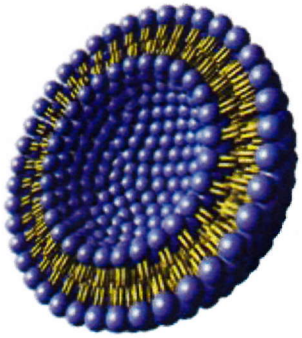

Fig. 1 Liposomes

The byword "liposome" has its origin from the Greek and means "fatty body" - and here the confusion begins: Which fat is a component of the liposomes? Liposomes are generally made of lecithin, at least the liposome described firstly in the literature consisted of phospholipids [A.D. Bangham; Adv. Lipid Res., 65-104, 1963]. However, lecithin for itself is a blend of quite different phospholipids that can be distinguished by its head groups and its fatty acid chains.

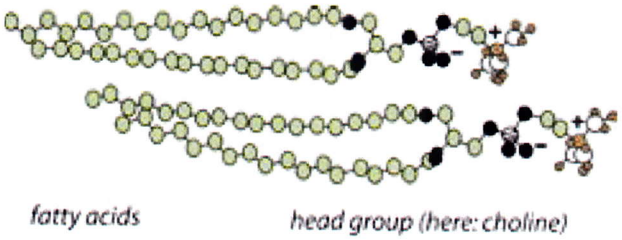

Fig. 2 Liposome Structure

2. Penetration of liposomes

[Dr. U. Schäfer, University of Saarbrücken]

Do all liposomes have the characteristic to deliver active substances into the deeper skin layers? What must a liposome look like to actually fulfil these claims?

To answer these questions, one investigated the liposomes' ability to penetrate independently of the lecithin composition.

For this, two different fluorescently labeled molecules – the hydrophilic carboxyfluorescein and the lipophilic rhodamin-PE – have been encapsulated into liposomes with the same size, the same loading and the same quantity of lecithin.

The decisive difference between these variable liposomes was the lecithin used for this purpose. Rovisomes consist of a high-quality phospholipid with a percentage of 80 % phosphatidylcholine. The fatty acids of these phospholipids are mainly unsaturated (linoleic acid) and provide the liposome with a flexible membrane. Furthermore, liposomes were produced from a very "cheap" lecithin with a small amount of phosphatidylcholine, which have, however, the feature of unsaturated fatty acids. The PL 90H-liposomes are based on a very high content of phosphatidylcholine (< 90 %) with hydrated, saturated fatty acids that strengthens the vesicle membrane.

The ethanolic solution and the three liposome-preparations were non-occlusively and ex vivo applied onto human skin obtained after cosmetic surgery. The experiment was carried out in Franz-type diffusion cells. One made sure that the acceptor compartment was filled with phosphate buffer below the skin to avoid over-hydration [T.J. Franz, Curr. Probl. Dermatol. 7, 58-68, 1978]. Three hours after application, the skin surface was cleaned and skin cylinders were punched. After cryo-fixation, pieces with a thickness of 10 μm were cut. The penetration of the marker-molecules were examined by confocal laser scanning microscopy.

Only the Rovisomes act as a carrier system that sets free the lipophilic as well as the hydrophilic marker in the epidermis, so

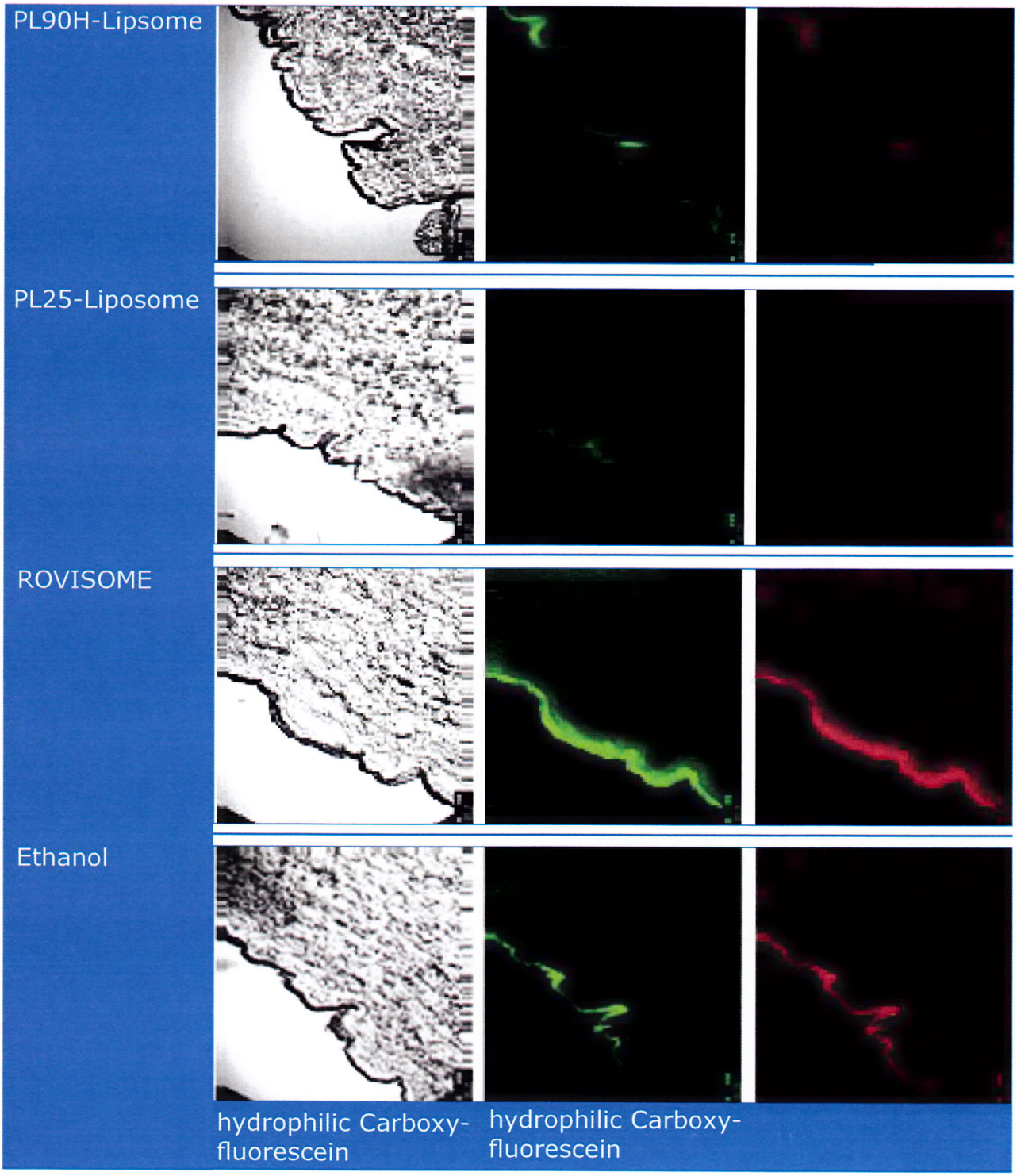

Fig. 3 Penetration of the marker molecutes examined by confocal laser scanning microscopy

that the dyes can even be detected in the dermis. In contrast, the PL 90H-liposomes with the rigid lipid membrane keep the fluorescent dye fixed on the skin surface. Other research groups describe the ability of flexible and fluid liposomes to transport active ingredients more into the skin increased compared to vesicles that are available in the crystalline phase [M. Kirjavainen et al., J Control Rel. 58, 207-214, 1999; B.A.I. van den Bergh et al., Int. J. Pharm. 167, 57-67, 1998]. Furthermore, owing to the high amount of ethanol (supports the penetration) the marker -solutions could color the stratum corneum. Liposomes produced from the "cheap" lecithin with a low content of phosphatidylcholine do not show any penetration-properties!

Noticeable during the literature-investigations, it was noticeable that for penetration studies with liposomes, almost all lipophilic markers, that are firmly tied into the vesicle membrane, were used. On studies with Rovisomes it could be shown that this carrier system is particularly suitable for the transport of hydrophilic active ingredients. Despite of the fluid membrane, a transmission-efficiency of about 80 % for water-soluble drugs can be achieved [G. Blume et al., SÖFW Journal 5, 298-301, 1997].

3. The stability of liposomes in finished formulations

[Dr. C. Johann, Wyatt GmbH, Woldert]

The AFFF (asymmetrical flow-field-flow-fractionation) is a new technique that enables the detection of liposomes in finished formulations [C. Johann und G. Blume, GIT Laborfachzeitschrift (special magazine for laboratory purposes) 4, 360-362, 1999].

In this technique, the sample is transported in a liquid flow from the injector to the detector and is separated. The separation is caused through the interactions with a field vertical to the transport direction. The closer a particle stays on the accumulation-wall (big vesicles), the slower it is transported in the parabolic flow-profile, accordingly it elutes later (small particles arrive first).

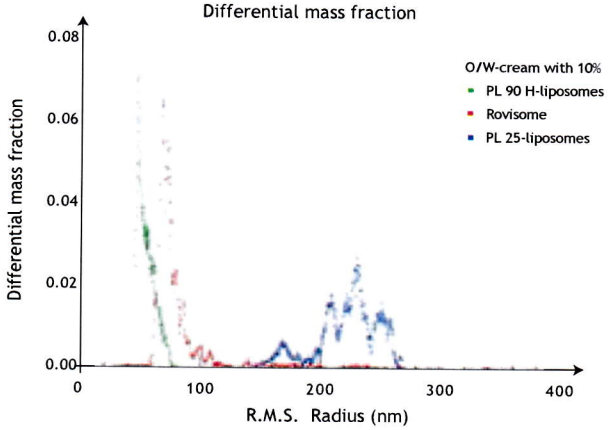

Fig. 5 Differential Mass Fraction

Ten percent of liposomes were stirred into O/W creams [MN-03-1198-560*], and the particle size of the vesicles were measured at once and after three-months storage at room temperature. In this experiment one also compared three liposome preparations with each other (Rovisome, PL 90H-liposomes and PL 25-liposomes).The very stable and rigid PL 90H-liposomes keep a constant vesicle-size in the finished formulation over the time.

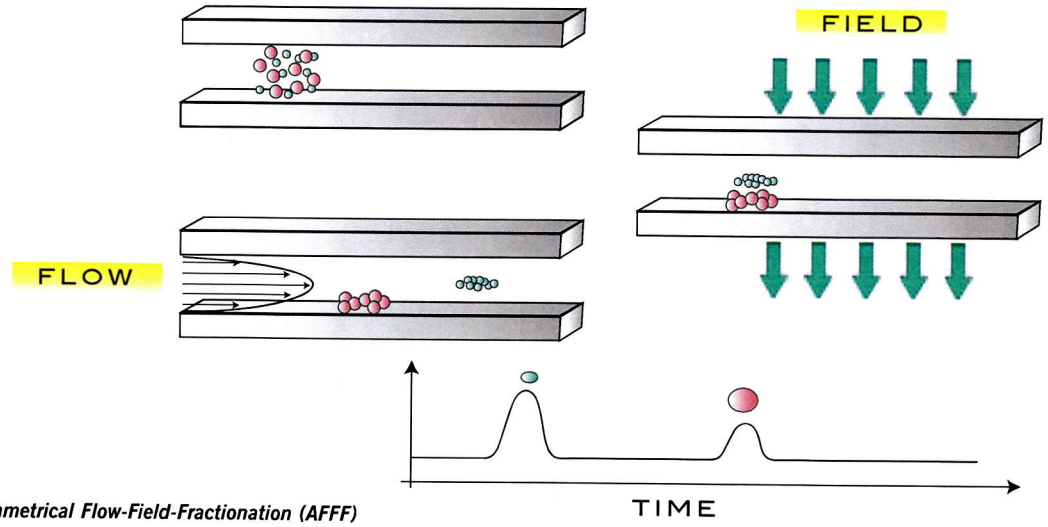

Fig. 4 Asymmetrical Flow-Field-Fractionation (AFFF)

The Rovisomes' size on the other hand (+ 70 µm) grows a little bit which is due to an increase of the water envelope bound on the membrane-surface. This hydrophilic surface delays the penetration of the Rovisomes from such a finished formulation into the skin in comparison with the application of an aqueous liposome-dispersion [G. Blume et al., SÖFW Journal 5, 298-301, 1997]. The "cheap" liposomes can definitely not be detected in the finished formulation. Particles bigger than 400 µm in diameter are found that lie in the area of fat drops. A similar result is obtainable from finished formulations without additives of liposomes.

* Formulation [INCI-Declaration]: Water, Hydrogenated Jojoba Oil, Steareth-2, Glycerin, PPG-15 Stearyl Ether, Hydrogenated Canola Oil, Dioctyl Adipate, Steareth-21, Dicaprylyl Ether, Alcohol, Shea Butter, Lecithin, Cyclomethicone, Polyacrylamide, C13-14 Isoparaffin, Laureth-7, Xanthan Gum, Sodium Hyaluronate, Preservatives

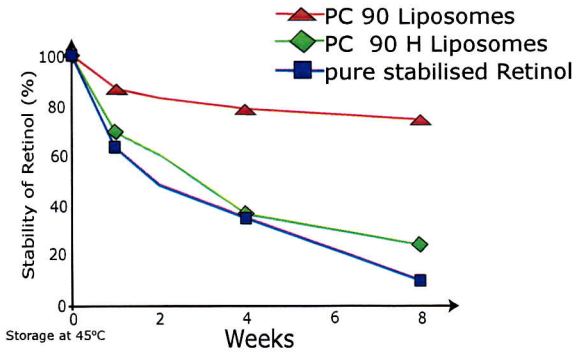

Fig. 6 Stability

4. Chemical stability of encapsulated components

Long-term stability of retinol in ROVISOME Retinol Moist was determined (flexible liposomes) and compared with a vesicle formulation made of hydrogenated phosphatidylcholine (rigid liposomes). All preparations were kept over eight weeks at 45 °C in a dark place and examined via HPLC.

5. Oxidative stability of ROVISOMEs

The oxidative stability was determined by measuring the rancidity (AOCS OM Cd 12b-92). The OSI (oxidative stability index) was > 150 hours at 110°C. The linoleic acid bound into the membrane of liposomes is much more resistant to oxidation compared to linoleic acid in triglycerides (soya bean oil).

6. Demonstration of the effectiveness of ROVISOMES

The effect of Rovisomes on the skin's moisture balance and its surface profile was determined in the course of a twenty eight-day study carried out on twenty two female test persons. There were significant differences in the unfilled liposomes, compared to the initial value. After twenty eight days, the moisture content of the skin had increased by 16.4 %, and its surface was 17.4 % smoother. In addition to this, the number of wrinkles was reduced by 6 %.

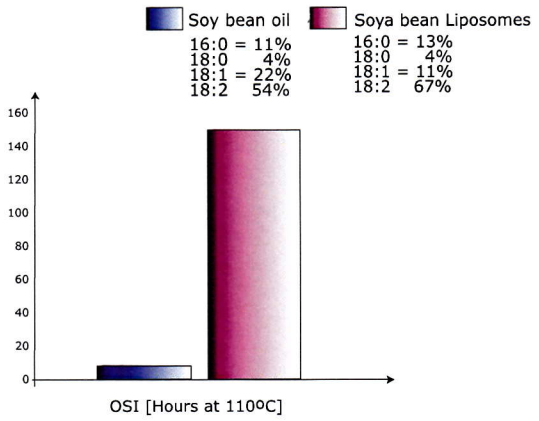

Fig. 7 Oxidative Stability of ROVISOMEs

7. Conclusions

The INCI declaration of the so-called "cheap-liposomes" and the Rovisomes are identical and are called "lecithin". However, in investigations it was clearly proven that these "cheap-liposomes" do not show any vesicle-stability and that they are unsuitable to carry active substances into the skin. Consequently, they do not correspond to their claims as "liposome-containing formulations", respectively "liposomes that are capable of penetrating, and enabling the transport of active substances into the skin".

Lipid vesicles, produced of the high-quality PL 90 H (hydrogenated lecithin) show an excellent stability in the finished formulation and have the ability to stabilize active ingredients. Liposomes with a rigid membrane on the other hand, do not transport the encapsulated substances into the epidermis, but fix the lipids as well as the active ingredients on the skin's surface and where, they act as a moisturizer.

Special designed liposomes, such as Rovisomes, have the characteristic to stabilize the integrated active substances in the vesicle dispersions and in the finished formulations as well. These liposomes also show a high stability in finished formulations and are able to carry the active ingredients out of the formulation into the deeper skin layers. In addition to this, Rovisomes are capable of smoothing the skin and of creating a significant reduction of wrinkles.