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Liposomes = Liposomes ?

Keywords: Liposome, Drug Carrier System, Penetration

Zusammenfassung

Liposomen sind mikroskopisch kleine Vesikel (Hohlkugeln), bestehend aus einer oder mehreren Lipiddoppelschichten, die sich um einen wässerigen Kern lagern. Sie werden in der Kosmetik als Transportsysteme, zur Stabilisierung von Wirkstoffen oder aufgrund ihrer feuchtigkeitsspendenden Eigenschaften eingesetzt.
Der Begriff »Liposome« kommt aus dem griechischen und bedeutet »Fett-Körperchen«. Und damit fängt die Verwirrung an. Welches »Fett« bildet den Bestandteil der Liposome? Im allgemeinen werden Liposomen aus Lecithin hergestellt, zumindest bestand das erste in der Literatur beschriebene Liposome aus Phospholipiden (A.D. *Bangham*; Adv. Lipid Res., 65-104, 1963). Lecithin selber ist aber schon ein Gemisch aus ganz verschiedenen Phospholipiden, die sich in den Kopfgruppen oder in den Fettsäureketten unterscheiden können.
Aber dem nicht genug. Zu den Liposomen zählen noch die Cerasome (Ceramide), Sphingosome (Sphingolipide), Nanosome (Phospholipide), Niosome (non-ionic surfactants) und ???-some. Alles »Fett-Körperchen«, die wegen ihrer verschiedenen Lipide unterschiedliche Eigenschaften aufweisen.

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1. What are liposomes?

Liposomes are microscopic small vesicles (hollow spheres), consisting of one or several lipid bilayers that surround a watery nucleus. They are used in the cosmetics as carrier systems and as stabilizer of active substances and regarding their moisturizing properties as well **(Fig. 1)**.

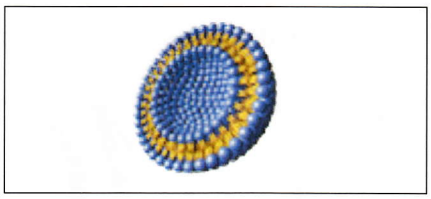

Fig. 1 Liposome

The byword »liposome« has it's origin from the Greek and means »fatty body" – and here the confusion begins: Which fat is component of the liposomes? Liposomes are generally made of lecithin, at least the liposomes described firstly in the literature consisted of phoshpolipids (*A.D. Bangham*; Adv. Lipid Res., 65-104, 1963). However, lecithin for itself is a blend of quite different phospholipids that can be distinguished in its head groups and its fatty acid chains **(Fig. 2)**.
However, that's not all! Beside the group of lipsomes there are also cerasomes (ceramides), sphingosomes (sphingolipids), nanosomes (phospholipids), nio- somes (non-ionic surfactants) and ???-somes. All of them are »fatty bodies« that show individual characteristics due to their different lipids.

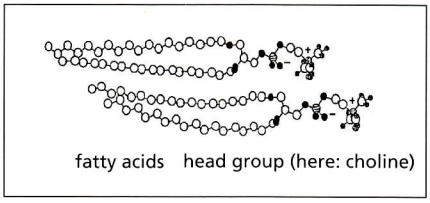

Fig. 2 Phosphilipids

2. Penetration of liposomes
(Dr. *U. Schäfer*, University of Saarbrücken)

In this article you will only be informed about liposomes made of lecithin. Not only the excellent moisturizing effects of this kind of liposomes will be reported but advertisements also more and more claim their properties as carrier system:

- »Liposomes are microscopic small hollow spheres filled with active ingredients. They are able to penetrate into deeper skin layers«.
- »Owing to their small size, they find their way into these skin layers in which the important processes of the regeneration take place«.
- »Liposomes have the unique capacity of transporting active ingredients. They are in a position to transport deeply into the epidermis«.
- »The pure vegetable liposomes produced of soy-lecithin penetrate particularly deep into the skin due to their structure similar to the skin. There they develop their skin smoothing effects«.
- »Liposomes are used as a carrier of active substances. They go through the skin and release their contents in the deeper skin layers of the upper skin, thus they not only act on the surface«.
- »The liposomal encapsulation enables the active substances to penetrate through the horny layer and to develop their effects there«.

– »Liposomes = lipid-like micro vesicles that penetrate as carrier for active substances into the upper skin, that spread out there and then enable the distribution of the active substances up to the deepness of the epidermis«.

Have all liposomes the characteristic to deliver active substances into the deeper skin layers? How must a liposome look like to fulfil these claims actually? To answer these questions, one investigated the liposomes' ability to penetrate in dependency of the lecithin composition.
For this, two different fluorescently labelled molecules – the hydrophilic carboxy-fluorescein 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 of 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 strengths 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 **(Fig. 3)**.
Only the Rovisomes act as a carrier system that sets free the lipophilic as well as the hydrophilic marker in the epidermis, so that the dyes even can 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 the active ingredients increased

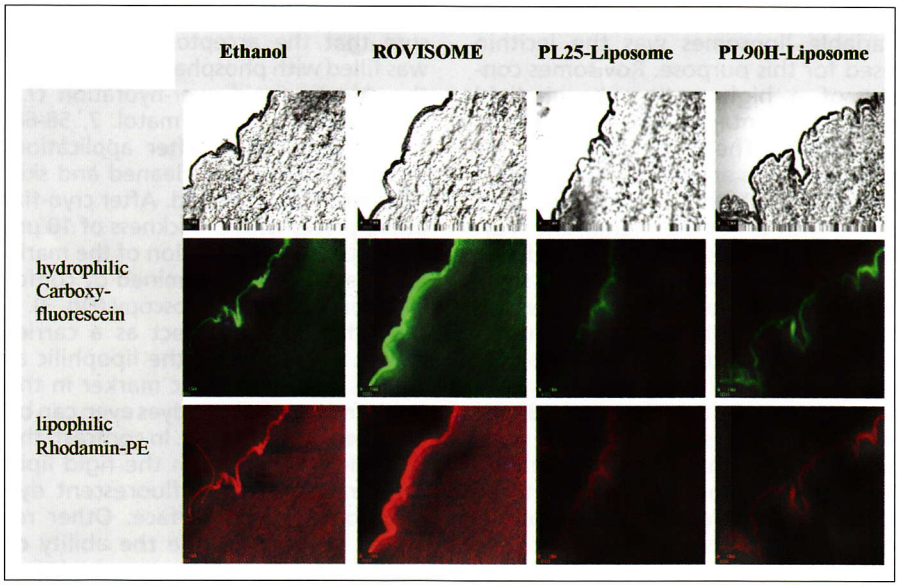

Fig. 3 Penetration of different liposomes

into the skin compared with 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 (that increases the penetration), the marker-solutions could colour the stratum corneum. Liposomes produced of the »cheap« lecithin with a low content of phosphatidylcholine do not show any penetration-properties!

Noticeable during the literature-investigations was that for penetration studies with liposomes nearly only lipophilic markers are used that are firmly tied into the vesicle membrane. On studies with Rovisomes it could be shown that these carrier system is particularly suitable for the transport of hydrophilic active ingredients. Despite of the fluid membrane, the 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-fractionation) is a new technique that enables the detection of liposomes in finished formulations (*C. Johann* und *G. Blume*, GIT Labor-Fachzeitschrift (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 nearer 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 at first) **(Fig. 4)**.

Ten percent of liposomes were stirred into O/W cremes (MN-03-1198-560*), and the particle size of the vesicles was measured immediately after this procedure and after a 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 constantly vesicle-size in the finished formulation over time. The Rovisomes' size on the other hand (+ 70 µm) grow a little bit 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 compari-

** Formulation (INCI-Declaration): Water, Hydrogenated Jojoba Oil, Steareth-2, Glycerin, PPG-15 Stearyl Ether, Hydrogenated Canola Oil, Dioctyl Adi- pate, Steareth-21, Dicaprylyl Ether, Alcohol, Shea Butter, Lecithin, Cyclomethicone, Polyacrylamide, C13-14 Iso-paraffin, Laureth-7, Xanthan Gum, Sodium Hyaluronate, Preservatives*

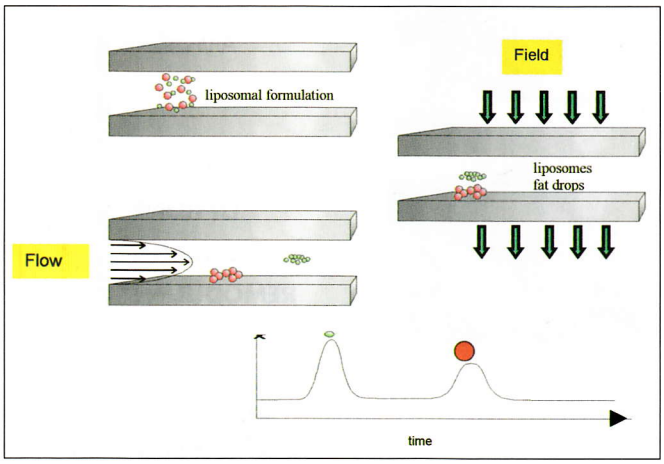

Fig. 4 Principle of AFFF

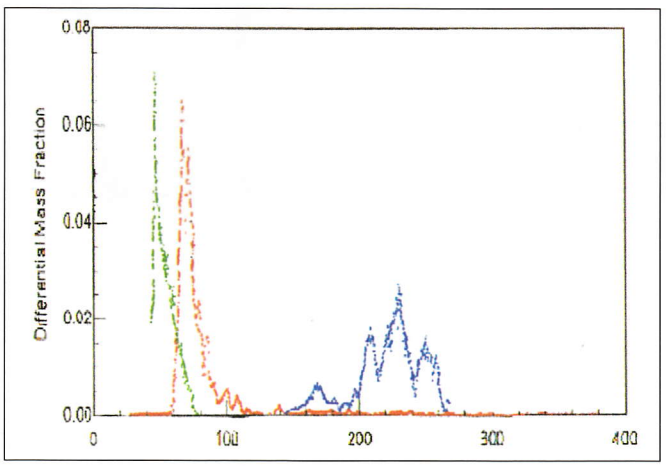

Fig. 5 Size distribution of different liposomes in a final formulation

son with the application of a aqueous liposome-dispersion (*G. Blume* et al., SÖFW Journal 5, 298-301, 1997). The »cheap« liposomes cannot be detected in the finished formulation definitely. Particles bigger than 400 µm in diameter are found to lie in the area of fat drops. A similar result is obtainable from finished formulations without additives of liposomes **(Fig. 5)**.

4. Stability of Retinol in liposomes

***(D.D. Verma* and Prof. *A Fahr* University of Marburg)**

In this experiment the chemical stability of pure retinol and two »liposomal« encapsulations with the same lipid composition was compared with help of reversed-phase HPLC (Methode nach *D.D. Verma*, Universität Marburg). Retinol was directly integrated into the lipid membrane – on the one hand encapsulated into Rovisomes, on the other hand it was incorporated into vesicles that – in contrast to the Rovisomes – contained the vitamin solved in oil (e.g. nanoparticles, nanosomes respectively nanospheres). Rovisome Retinol was also incorporated into a O/C-creme (*MN-03-1198-560) and checked on the stability of the finished formulation **(Fig. 6)**.
In this example it becomes clear that not only the lecithin composition is decisive but also the kind of the encapsulation. Indeed one can encapsulate a higher concentration of lipophilic active ingredients into the nanoparts, however, due to the »half« bilayer, the membrane becomes unstable and, as a result of this, the retinol will be degraded faster.
The incorporation of Rovisome Retinol into this O/W-creme leads to a further slight increase of stability of the vitamins in the formulation. The stability of the retinol in the nanoparts on the other hand could not be increased by incorporating them into the cream.

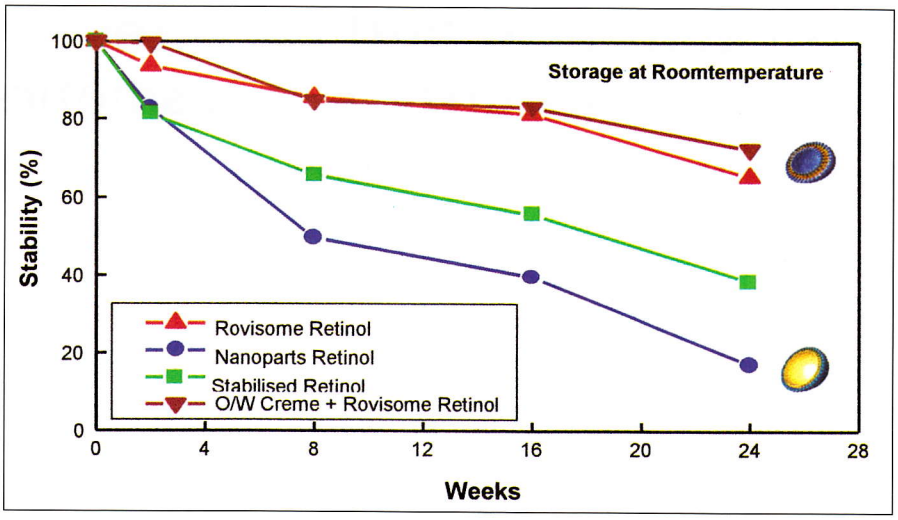

Fig. 6 Stability of Retinol encapsulated or in its free form

4. Conclusion

The INCI declaration of the so-called »cheap-liposomes« and the Rovisomes is identical and 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 to penetrate, and that enable 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, transport the encapsulated substances not into the epidermis but fix the lipids as well as the active ingredients on the skin's surface and there, 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 (*G. Blume* et al., Euro Cosmetics 3, 30-32, 2000). In addition to this, Rovisomes are capable to smooth the skin and to cause a significantly reduction of wrinkles (*G. Blume* und *E. Teichmüller*, Cosmetics and Toiletries Manufacture Worlwide, 135-139,1997).
Conclusion: The subject »lipsosomes« includes several »lipid-bodies« with the same INCI-declaration, that, however, differ significantly in price, in quality and in their properties. Therefore, they should be investigated strictly with regard to ability and marketing claims.

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No !

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