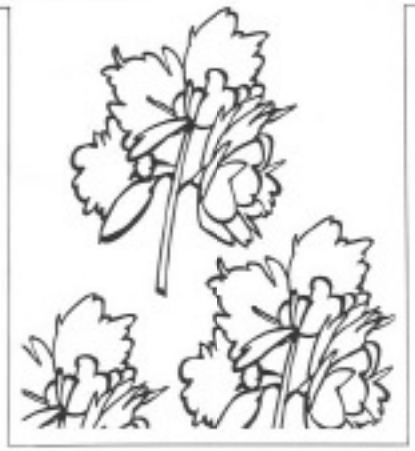

Liposomes
in Dermatological Preparations
Part I

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Liposomes or liposomal aggregation may have played a role in the formation of the first cell membrane on Earth. Immediately after the discovery of liposomes by Bangham,[1] there was intense research in the pharmaceutical industry concerning their parenteral application, but without a decisive breakthrough. It was only with the success of the first liposomal cosmetic called "Capture" that it became evident that the possibilities for the application of artificial vesicles could be achieved in practice in the market place.

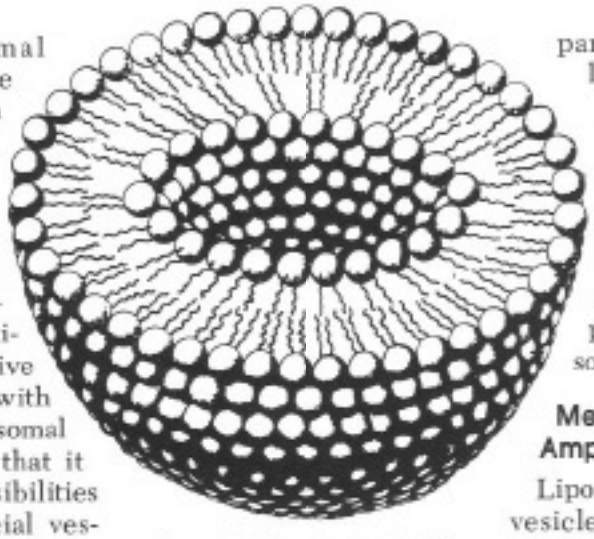

Figure 1. Liposome model.

Interestingly, the boom in new liposomal cosmetic preparations that followed and continues unabated today has of itself rekindled the development of liposomal dermatologicals and liposomally packaged pharmaceuticals. Naturally such developments take longer in the pharmaceutical field.

We may assume that topical applications will be of great importance in the immediate future. It therefore is appropriate not to consider cosmetic and dermatological preparations separately. This is particularly true because the larger companies in the cosmetics industry, which do their own research, tend ever more frequently to adopt pharmaceutical standards. Furthermore, the boundaries between cosmetics and dermatology are very fluid, particularly in the field of liposomal formulations.

Membrane-Forming Amphiphiles

Liposomes are defined as spherical vesicles, the membranes of which consist of a bilayer of amphiphilic molecules. The lipophilic tails orient to the middle of the bilayer. The polar heads are directed to the inside of the vesicle and to its outer surface (Figure 1).

Most cosmetic and pharmaceutical liposomes are composed of various phospholipids of natural, semisynthetic and synthetic origins, with the major component usually being phosphatidylcholine (Figure 2). Minor components can include phosphatidylethanolamine, phosphatidylinositol and phosphatidic acid. A distinction is made between

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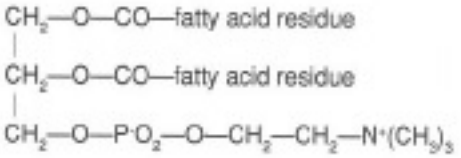


Figure 2. Phosphatidylcholine

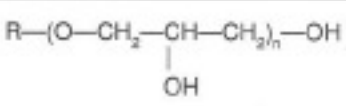


Figure 3. Polyglycerol ether

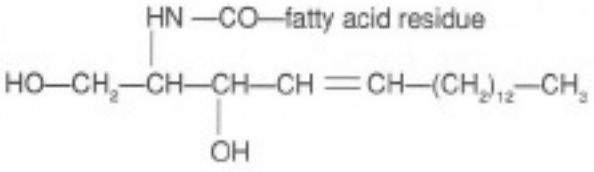


Figure 4. Ceramide

unsaturated, partially hydrogenated and hydrogenated phospholipids according to the composition of the fatty acids. There are reviews of phospholipids and liposomes by H. P. Fiedler in the most recent edition of the *Lexikon der Hilfsstoffe für Pharmazie, Kosmetik und angrenzende Gebiete*[2] and in references 3, 4 and 5.

Niosomes are, from a chemical point of view, special cases of liposomes. The main components of niosomes are ethoxylated fatty alcohols and synthetic, linear or branched chain polyglycerol ethers (Figure 3), with n being a number between 1 and 6 and R being a linear or branched-chain saturated hydrocarbon residue such as hexadecyl.

These polyglycerol ethers are members of the group of nonionic tensides (niotensides). They are usually formulated with cholesterol (frequently in the ratio 1:1 (w/w) and with dicetyl phosphate, and contain other formulation components for the preparation of niosomes.[6,7]

Sphingolipids are derived from the chemical sphingosine. In the form of their natural derivatives, ceramide (Figure 4), the cerebrosides and the sphingomyelins, they can also form vesicles ("sphingosomes"). These usually are present in the form of mixtures, and have differing compositions depending on their sources.

Other bilayer-forming amphiphiles include dialkyl phosphates and N,N-dimethyl-N,N-dialkylammonium salts.[8] The dicarboxylic acid diesters of sucrose[9] constitute an interesting group of substances. Depending on the chain length of the

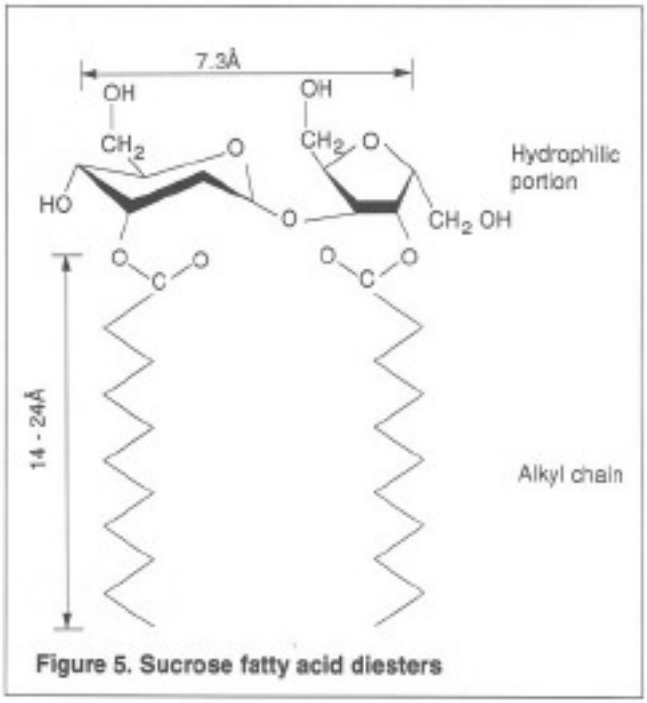


Figure 5. Sucrose fatty acid diesters

esterified carboxylic acids, they form vesicles with mean particle sizes of 290 to 1080 nm (Figure 5, after Y. Ishigami and H. Machida, 1989).

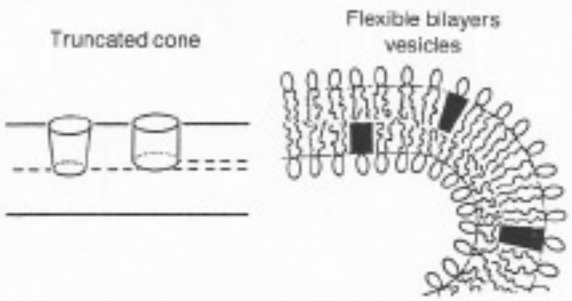
In general the different chemical components of liposomes can be divided into these groups:

- Phospholipids
- Non-phospholipids
- Polymerisates[6]
- Mixtures of different raw materials
- Natural raw materials
- Semisynthetic raw materials
- Synthetic raw materials
- Positively-charged starting compounds (amines, quaternary compounds)
- Negatively-charged starting compounds (dialkyl phosphates, carboxylic acids etc.)
- Neutral starting compounds (betaines, niotensides, diglycerides, ceramides)

All membrane-forming amphiphiles possess a very low critical micelle concentration (cmc) of ca. 10^{-8} mol/l and less. For example, the cmc of dipalmitoylphosphatidylcholine is 4.6×10^{-10} mol/l;[10] compared with ca. 10^{-3} mol/l for normal surfactants. The low critical micelle concentrations of these substances are probably an important reason for the kindliness of these substances to skin, because the aggressivity of a surface-active substance generally is related to the concentration of free molecules.

When present in membranes these molecules usually take on the shape of slightly tapered truncated cones or cylinders[11] (Figure 6, after Israelachvili et al., 1980). Amphiphiles that deviate from this shape require higher quantities of stabilizers, such as cholesterol.

The names given to commercial liposomal products are often very imaginative. There are Brookosomes, Dermosomes, Glycosomes, Lipocutin,



Double-chain lipids head area: large fluid chains (ecithin, sphingomyclin, phosphatidylserine in water, phosphatidylglycerol, phosphatidic acid, disugardiglycerides, some single chained lipids with very small, uncharged head groups)

Figure 6. Arrangement of liposome components in the vesicle (Israelachvili et al., 1980)

Lipodermin, Liposome Concentrate E-10, Natipides, Niosomes, Sphingosomes, Super-Lipodermin, etc. The concentrations and ingredients differ very greatly in measurable parameters such as:

- Ratio of the encapsulated volume to the total volume (V/V in %)
- Number of the dispersed vesicles per volume unit (n/V)
- Ratio of the weight of a liposomal dispersion used to the final formulation (w/w in %)
- Ratio of the liposomal raw material to the formulation (w/w in %)

Liposomes are offered with or without preservatives, loaded or unloaded, with or without stabilizers, in the form of aqueous dispersions or as powders for dispersion in water.

From the point of view of availability, standardization and available literature data, phospholipids take first place among the liposome raw materials and are followed by niosome raw materials.

General Aspects of Liposomal Formulation

Liposomes can be classified as unilamellar (single-shelled) oligolamellar (several-shelled), or multilamellar (many-shelled), and homogeneous (with a narrow size distribution) or inhomogeneous (with a wide size distribution) liposomes, with diameters ranging from 15 to 3500 nm. The usual manufacturing processes depend on the application of high-energy homogenizers and are divided in two important steps:

1) Hydration of the liposomal raw material and formation of inhomogeneous vesicles, and
2) Making the vesicles uniform.

In a manner similar to biological cells, liposomes can store water soluble substances in their interiors and lipophilic and amphiphilic substances in their membranes (loaded liposomes). The following parameters of liposomes have to be taken into consideration when formulating topical applications:

- Chemical composition,
- Mean vesicle size,
- Lamellarity,
- Shape (spheres, aggregates, propellers),
- Surface charge,
- Phase transition temperature,
- Critical micelle concentration,
- Place of entrapment (inside the vesicles, in the membrane, on the outer surface of the vesicles),
- Species of entrapped agents (hydrophile, amphiphile, lipophile),
- Capacity of storage, and
- Homogeneity.

In the preparation of final products, further variables and unknowns must be considered:

- Chemical composition,
- Physical properties,
- Chemical and physical stability,
- Compatibility with other ingredients,
- Penetration of the liposomes or their components into or through the skin,
- Demonstration of efficacy,
- Pharmaceutical or cosmetic effect,
- Tolerance,
- Comparisons with classical formulations, and
- Advertising slogans.

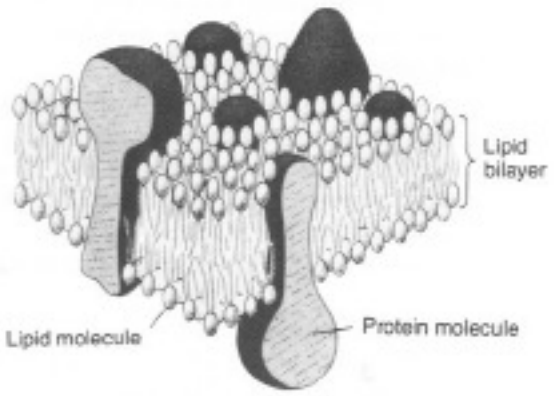


Figure 7. Model of cell membrane

It is important to point out that liposomes are not only a new type of formulation. They also have the character of active dermatological and cosmetic components because of the properties of the amphiphiles used. The vegetable phospholipids should be mentioned here because of their high content of linoleic acid and α-linoleic acid (essential fatty acids).

The following potential properties of liposomes can, therefore, be exploited in dermatological and cosmetic preparations:

- New type of formulation with new physical properties,
- Transport system for dermatological and cosmetic agents,
- Increased efficacy of dermtological and cosmetic agents,
- Intrinsic dermatological and cosmetic effects (empty liposomes),
- Transport system for intrinsic dermatological and cosmetic effects,
- Transport of essential substances into the skin,
- Deep effects,
- Advertising slogans, and
- Others.

Before deciding on the final composition of a liposomal product, the potential mechanisms of action of liposomes should be taken into consideration.

Empty Liposomes

The complex nature of the properties of liposomes can best be demonstrated by liposomes made up of polyunsaturated phospholipids on the basis of a membrane model.

The human body is composed of about 6 x 10^{13} cells, of which the membranes make up an extraordinarily large surface area. For example, the area of the tissue cell membranes amounts to about 35,000 m^2.[12] Proteins, glycoproteins, glycolipids and cholesterol are embedded in their phospholipid bilayers[13] (Figure 7, after A. Bruce et al., 1986). This

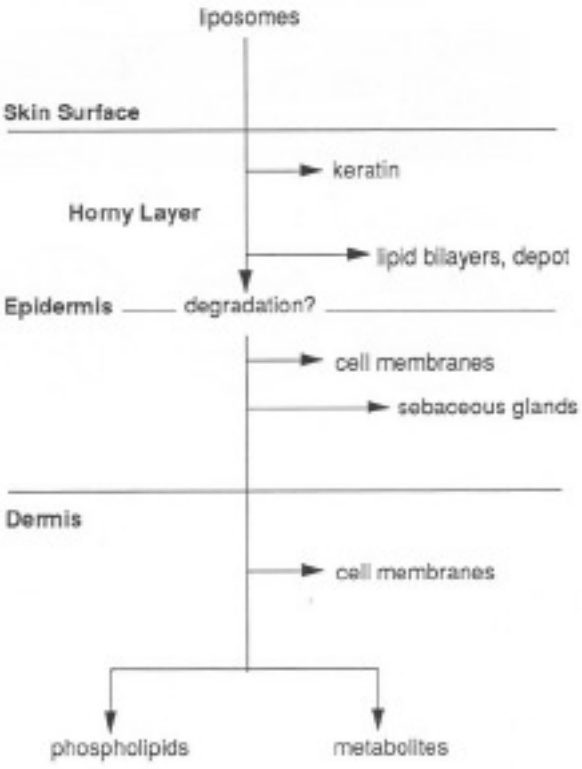


Figure 8. Potential effect of liposomes on the skin (simplified schematic).

structure is stabilized by very different types of interactions: ionic interactions, hydrogen bonds, polar interactions, charge-transfer interactions, and Van der Waals forces.

The same interactions come into play when phospholipids—and this applies especially to liposomes—are applied to the skin in the form of dermatologicals or cosmetics. They readily form associations with the proteins, carbohydrates and lipids to be found at the surface of the skin and in the skin. This explains the three phase potential effect of the phospholipids (Figure 8).

In the first phase, the phospholipids are bound superficially to the keratin of the horny layer (cf. interactions with membrane proteins).[14] This process is responsible for the spontaneous feeling of the skin being coated after the application. This film lipophilizes the surface of the skin.

The film cannot be removed with water, and only slowly with detergents. As a result of its slightly occlusive effect, the film reduces transepidermal water loss and so amplifies the barrier function of the skin, which is of particular advantage for dry skin.[15] This strong affinity to keratin, however, results in the destruction of some of the liposomes.

The same thing probably occurs at the lipid bilayers of the horny layer. These complex layers are formed as an "intercellular cement" by the keratinosomes.[16,17] They carry out an important barrier function and exert a disproportionate effect on

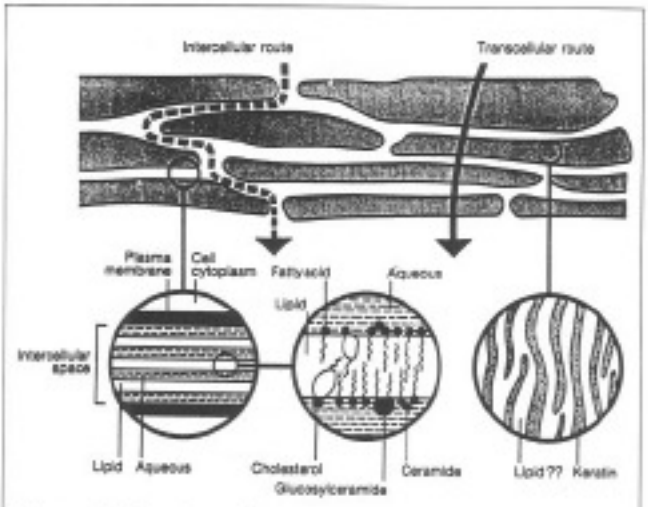


Figure 9. Structure of stratum corneum

transepidermal water loss. Figure 9 shows the lipid bilayers of the stratum corneum.[18]

The property of the liposomes to support the structure and the restoration of the lipid bilayers supports the present opinion, that liposomes are not moisturizers in the common sense, but are a *source of bilayers* in general. On the other hand, the result of the support is not an occlusive effect similar to paraffin oil. The mechanism of action can be described here more by the comparison of moisture regulation of a respiration-active rain coat with the impermeability of a respiration-poor rain coat. Whether the water stored in the liposomes plays a role is not yet clear.

It is important to understand these mechanisms to decide whether to aim for only these effects with a low dose level or whether to aim to produce further effects by means of a higher dose level (see Part II of this article, to be published in July). Most liposomal cosmetics are likely to affect the horny layer by means of a phospholipid-keratin interaction and by a deposition in the lipid bilayers.

In the second phase, the unbound phospholipids of the liposomes are probably introduced into the deeper layers of the skin. Here again, the cell membrane origins of the phospholipids make themselves felt, for they are rapidly taken up again by the cell membranes. It was demonstrated by human keratinocytes in vitro that an exogenous addition of soya phospholipid liposomes is very rapidly internalized, with a fluidization of the membrane being observed.[19]

Whether this mechanism actually involves the direct uptake of liposomes which is discussed so often,[20] or only occurs after their breakdown into individual phospholipid molecules or even their degradation into individual components, is not as yet clear. But the exact mechanism is probably not important for the "empty" liposomes being discussed here.

What has been established is that polyunsaturated vegetable phospholipids (containing linoleic and α-linoleic acids) increase the fluidity and permeability of the membranes.[21] The metabolism of the cells is activated appropriately.

The absorption of unsaturated phospholipids (nonliposomal and liposomal) through the skin had been demonstrated by means of radioactive labelling.[22] Saturated liposomal phospholipids with shorter fatty acid chains (DPPC type) appear to remain in the horny layer.[23] This also applies to niosomes.[6]

These findings are not necessarily contradictory, because not only were other phospholipids and liposomal raw materials used, but other types of vesicles were also used, which were often not adequately characterized. The vesicle size appears to be of great importance in this respect.

Similarly varying behavior is found in the absorption of phospholipids from the gastrointestinal tract. Some specific phospholipids are evidently absorbed intact,[24] while most phospholipids are taken up by means of the well-known deacylation-acylation process.

In the third phase, chemically bonded linoleic acid in polyunsaturated phospholipids possibly supplements the function of the sebacous glands (Figure 8). Some free linoleic acid is certainly produced by partial hydrolysis of the phospholipids taken up into the horny layer, and distributes itself as such in the epidermis.

The body can also use the essential fatty acids not only as a source of energy by oxidative degradation but also to synthesize further highly unsaturated fatty acids.[25]

Triglycerides and phospholipids of mammals contain chemically bonded linoleic acid. The content depends on the sort of tissue. Systemic effects certainly can be excluded in any possible topical absorption; decades of experience with phospholipids (lecithins) and highly unsaturated native oils confirm this view.

One is entitled to wonder whether there is a possibility here for local therapy of various skin complaints that have been associated directly with deficits of the metabolites named—especially since phospholipids and lecithins have no known side effects. Lecithin has been awarded GRAS status (Generally Recognized As Safe) by the US Food and Drug Administration (FDA) and is registered for use in pharmaceutical, cosmetic and food applications.[26]

It is not known how far the qualitative effect of sphingolipid-based vesicles on the skin differs from liposomes based on vegetable phospholipids. It can be assumed that the sphingolipids and phospholipids exert many common effects and that both are incorporated into the lipid bilayers of the horny layer. Because of their chemical composition, it is certain that the intrinsic effects of the essential fatty acids will be more or less absent.

On the other hand, sphingolipid composition is very similar to that of the lipids of the horny layer. Their external application may restore the natural functions of fatigued skin. What raw material or raw material combinations finally find application will depend on the aims intended and the demonstration of efficacy.

In this context, it should be mentioned that liposomes can be prepared starting with the main compounds of the bilayers of the horny layer, ceramides, cholesterol, palmitic acid and cholesterol sulfate, by use of ultrasound.[27] This is the reverse of the principal liposomes being a source of bilayers.

lipid bilayers ⟷ liposomes

It must be emphasized once again that the "mechanisms of actions" described here of empty liposomes on and in the skin are still open to many questions. Further biological investigation will be required to demonstrate the soundness of this concept for the dermatological field.

Until now there have been no exact results concerning the penetration of intact topically applied liposomes through the skin into the living tissue. Neither has it been demonstrated whether suitable "classical" phospholipid formulations exhibit comparable effects. This also applies, in particular, to the penetration-enhancing effect of loaded liposomes.

What has been demonstrated is that "comparable" classical phospholipid formulations are very difficult to produce because the "normal state" for phospholipids is, by their nature, in the form of liposomes.

This article will conclude in the July 1990 issue of Cosmetics & Toiletries.

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