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# LIPOSOMES IN DERMATOLOGICAL PREPARATIONS

**H. Lautenschläger**
Neusser Gasse 50, D-5024 Pulheim - Stommeln, Federal Republic of Germany

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## Synopsis

It was attempted to provide a simplified overview of a very rapidly growing field in cosmetic dermatology and to put it into some sort of context, summarizing some of the most important results concerning the field of liposomal dermatologicals and cosmetics.
1. Liposomes are a very many–sided topic, which cannot be treated with a routine formulation.
2. Liposomes are formulation and active ingredient in one.
3. The quality of the effect depends on the dose.
4. The liposomal potential is dependent on other ingredients in the formulation.
5. Liposomes have both a dermatological and a cosmetic justification.
6. The primary topic in the dialogue between the raw material manufacturers and the manufacturers of the preparations must be the working out of criteria for the evaluation of liposome-containing dermatologicals and the setting of technological standards

## Riassunto

Si é cercato di dare un panorama aggiornato dei problemi riguardanti la Dermatologia Cosmetologica riassumendo i risultati più importanti ottenuti nel settore dei liposomi quali vettori di utilizzo sia dermatologico che cosmetico.
1. I liposomi rappresentano un mezzo topico particolare che non può essere utilizzato per tutte le formulazioni usuali.
2. I liposomi sono nello stesso tempo veicoli e principi attivi.
3. La qualità dell'effetto é sempre dose-dipendente.
4. L'efficacia dei liposomi é strettamente correlata con tutti gli altri ingredienti che compongono la formulazione.
5. I liposomi sono adatti sia all'uso dermatologico che cosmetico.
6. Si deve stabilire un più stretto collegamento tra produttori di materie prime ed utilizzatori per valutare attentamente le caratteristiche standard del liposoma quale prodotto finito.

## Membrane-forming amphiphiles

Liposomes are defined as spherical vesicles, whose membranes consist of a bilayer of special amphiphilic molecules. (Fig. 1)

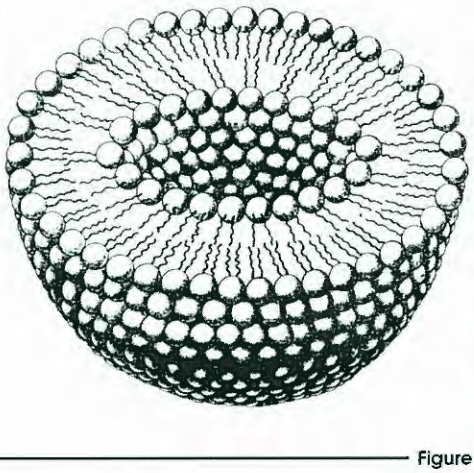

Figure 1

*Liposome model*

The liposomes generally referred to are composed of various phospholipids of natural, semisynthetic or synthetic origins, with the major component usually being vegetable phosphatidylcholine (Fig. 2):

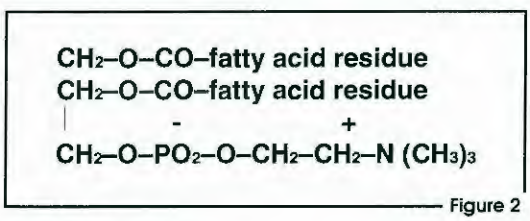


Figure 2

*Phosphatidylcholine*

Niosomes are, from a chemical point of view, special cases of liposomes. Besides ethoxylated fatty alcohols the main components of niosomes are synthetic polyglycerol ethers (Fig. 3).

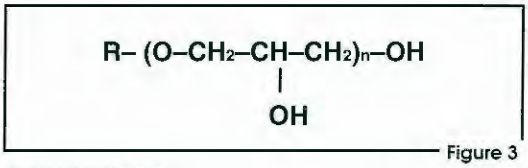


Figure 3

*Polyglycerol ether*

Other important vesicle forming agents are ceramides and sphingolipids in general.

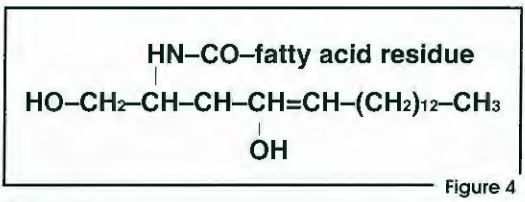


Figure 4

*Ceramide*

The dicarboxylic acid diesters of sucrose (1) constitute also an interesting group of substances (Fig. 5)

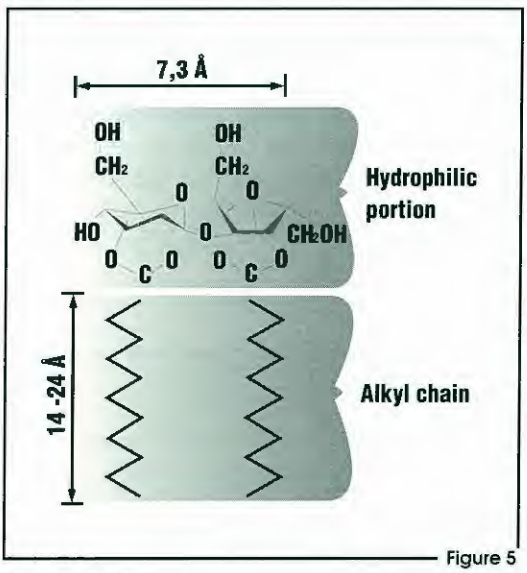


Figure 5

*Sucrose diesters*
*(Y. Ishigami and H. Machida - 1989)*

All membrane-forming amphiphiles possess a very low critical micelle concentration (cmc) of ca. $10^{-8}$ mol/l and less 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, since the aggressivity of a surface–active substance is generally related to the concentration of free molecules.

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

## The "mechanism of action" of nonloaded ("empty") liposomes

The complex nature of the properties of liposomes can best be demonstrated for liposomes made up of polyunsaturated phospholipids.
Like phospholipids interact with proteins, glycoproteins, glycolipids and cholesterol in the cell membranes (2) (Fig. 6):

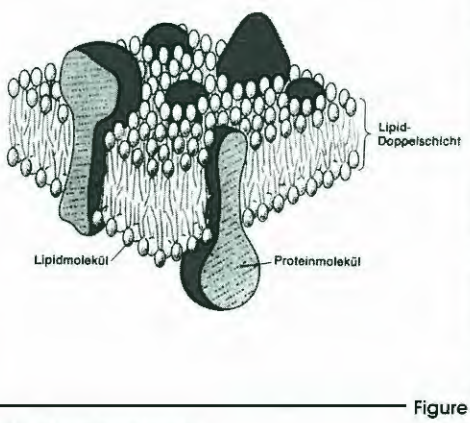


Figure 6

*Model of cell membrane (A. Bruce et al. - 1986)*

the same interactions come into play when phospholipids — and this applies especially to liposomes — are applied to the skin, i.e. they readily form associates with the proteins, carbohydrates and lipids to be found at the surface of the skin and in the skin. This explains the 4–phase potential effect of the phospholipids (Fig. 7).

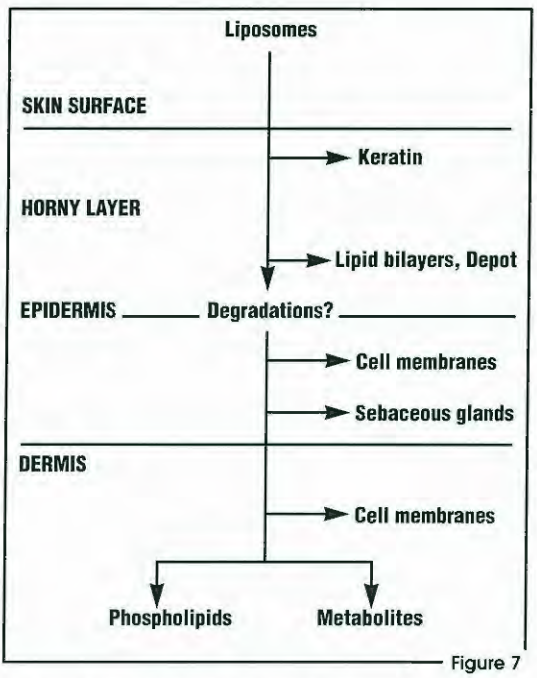


Figure 7

*Potential effects of liposomes on the skin (schematically simplified)*

1. In the first phase the phospholipids are bound superficially to the keratin of the horny layer (3) (cf. 4). This process is responsible for the spontaneous feeling of the skin being coated after the application. This film lipophilizes the surface of the skin and the film cannot be removed at all with water and only slowly with detergents.
However, this strong affinity to keratin results in the destruction of some of the liposomes. The same thing probably occurs at the lipid bilayers of the horny layer. There layers which are formed as an "intercellular cement" by the kerati-

nosomes (5) and whose composition is very complex (6) carry out an important barrier function and exert a disproportionate effect on inibition of transepidermal water loss.
It is important to realize this in order to decide whether to aim for just a support of the mentioned functions of the horny layer with a low dose level or whether to aim to produce further effects by means of a higher dose level (see below). 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.
2. In the second phase the remaining unbound phospholipids, or possibly liposomes, are probably introduced into the deeper layers of the skin (7).
Here again the origins of the phospholipids observed.
Whether this mechanism actually involves the direct uptake of liposomes (9), which is discussed so often, or only occurs after their breakdown into individual phospholipid molecules or even their degration into individual components is not as yet clear.
The penetration of the phospholipids has been confirmed in a study made in the Institute für medizinische Balneologie und Klimatologie of the University of Munich (10) (11) (Figs. 8, 9).
In this study a liposomal concentrate which was loaded with monoclonal antibodies was applied to porcine skin in vivo. After 40 minutes it was possible to demonstrate the presence of the antibody complex in both the epidermis and the dermis by specific coloration (APAAP method) (Fig. 8, reddish coloration). The antibody alone

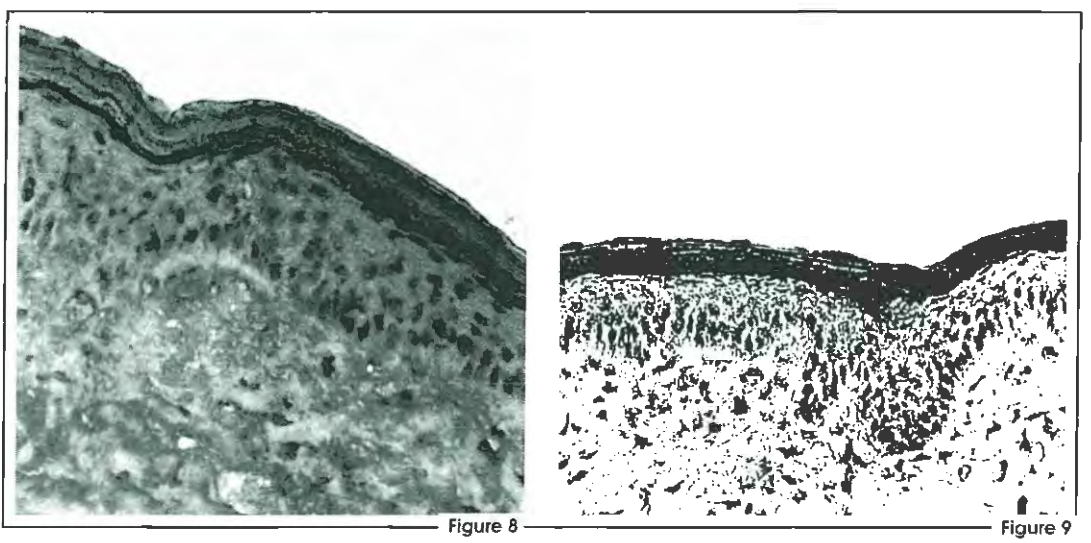

Figure 8 Figure 9

*Figs. 8 and 9: Sections through porcine skin, magnified ca. 280–fold.*
*Fig. 8: Liposomal formulation with antibodies; Fig. 9: Control without liposomes (C. Artmann and H. Pratzel - 1988)*

from membranes make themselves felt, for they are rapidly taken up again by the cell membranes. Bonnekoh et al. (8) have been able to demonstrate witb human HaCaT keratinocytes in vitro that an exogenous addition of soya phospholipid liposomes is very rapidly internalized, with a fluidization of the membrane being cannot penetrate the skin (Fig. 9).
The penetration of polyunsaturated nonliposomal phospholipids into the skin had been demonstrated earlier by means of radioactive labelling (12).
3. In a third phase the chemically bonded linoleic acid in polyunsaturated phospholipids can

possibly supplement the function of the sebaceous glands (Fig. 7). Some of the 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.

It is known that undersupply of the sebaceous glands with essential fatty acids leads to increased blackhead and pimple formation (13).

4. In a fourth phase the body can utilize the essential fatty acids not just as a source of energy by oxidative degradation but also to synthesize other polyunsaturated fatty acids (Fig. 10).

Thus, for example, linoleic acid released by phospholipases can lead via gamma–linolenic acid (6, 9, 12–C18:3) and dihomo–gamma–linolenic acid (8, 11, 14–C20:3) to arachidonic acid (5, 8, 11, 14–C20:4) with its metabolites and alpha–linolenic acid (9, 12, 15– C18:3) via eicosapentaenoic acid (5, 8, 11, 14, 17–C20:5) to docosahexaenoic acid (4, 7, 10, 13, 16, 19–C22:6) and its metabolites (14).

Systemic effects can certainly be excluded in any possible topical absorption; the decades of experience with phospholipids (lecithins) and highly unsaturated native oils confirm this view. Lecithin has been awarded GRAS status (generally recognized as safe) by the FDA and is registered for use in pharmaceutical, cosmetic and food applications (15).

To summarize, it must be emphasized once again that the "mechanisms of action" described

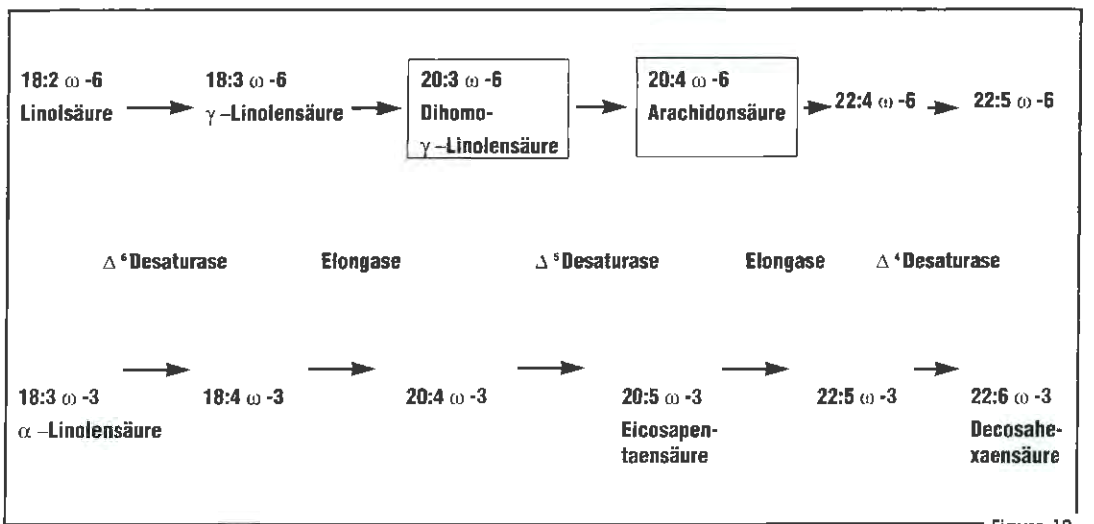


Figure 10

*Metabolism of linoleic acid and alpha-linolenic acids (S.Fischer-1987)*

here of empty liposomes on and in the skin are still subject to many open questions and that further biological results will be required to demonstrate the soundness of this concept for the dermatological field.

Until now there are 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. But what has been demonstrated is that "comparable" classical phospholipid formulations are very difficult to realize, since the "normal state" for phospholipids is of their nature in the form of liposomes.

## Loaded liposomes

When "loaded" liposomes are employed for topical application the effects are those of the phospholipids and of their "load".
The storage basically occurs at two sites: Hydrophilic substances in the aqueous interior of the liposome and lipophilic and amphiphilic substances in the membrane.
In the case of water–soluble substances inside the liposomes, losses by leakage must be expected, particularly of low molecular weight substances. However, this leakage can be counteracted by ensuring that the outside phase contains similar concentrations to those enclosed in the liposomes. It should be remembered in this context that even a 10% dispersion of liposomes (calculated as dry residue) represents a very tight packing of the liposome spheres. The following picture is an electron micrograph of a 1% (!) liposome dispersion (Fig. 11).

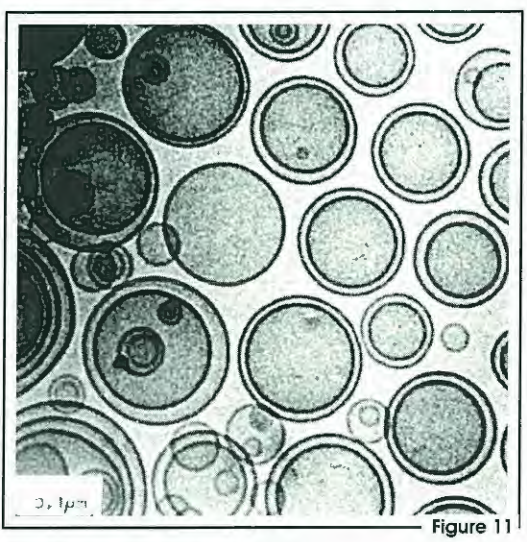

Figure 11

*Cryofixed liposomes; 1% dispersion*

Thus, separation from the water–soluble substances present in the outside phase is usually unnecessary and also too expensive in most cosmetic and dermatological applications.

It is the purpose of loaded liposomes, in addition to exerting their intrinsic effects, to transport their loads to the site where they are to exert their cosmetic or dermatological effects (Fig. 12). If the horny layer is the target then the distribution behaviour in this layer has priority. If transport is to take place into a deeper layer of the skin then the penetration–enhancing properties of the liposomes, respectively of their phospholipid components, are to the fore. In the case of steroids, retinoids etc., which can only exert their effect after appropriate absorption, this is the most important prerequisite along with a certain depot effect of the horny layer.

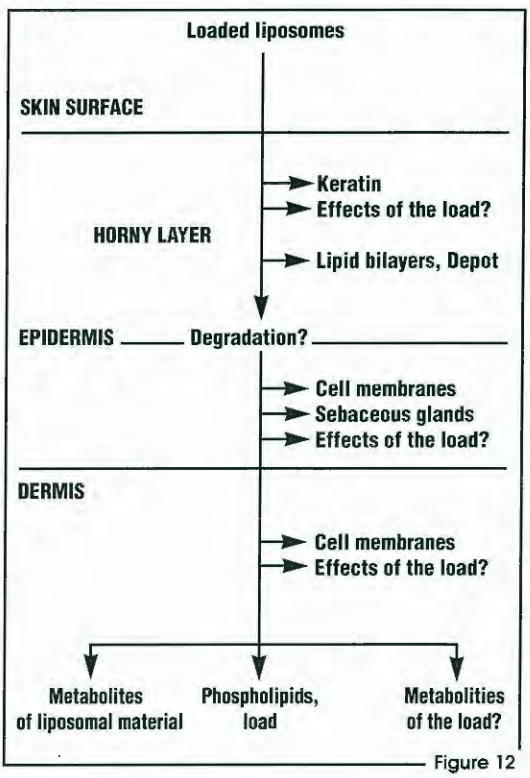


Figure 12

*Potential effects of loaded liposomes in the skin (schematically simplified)*

## Dermatological applications of liposomal formulations

The most important groups of substances which are being investigated at the moment for the local treatment of dermatological disorders with the aid of liposomal systems are probably:
Antimycotics (and topical antibiotics in general);
antiseptics should perhaps also be included here.
Corticosteroids
Retinoids
Another potential field of application are liposomal bath oils with dermatological activities:
1. The treatment of large areas of skin is very simple.
2. The drying–out of the skin, which is usually a problem in such preparations embloying normal surfactants, is reduced.
The fields of wound healing and, in particular, treatment of sun damage (sunburn) must also be mentioned.

## Cosmetic applications of liposomal formulations

The following types of preparation are to the fore:
1. Skin–care preparations with empty or moisturizer–loaded liposomes. Further potential effects are skin smoothing and supplying linoleic acid to the sebaceous glands.
2. Liposomes loaded with other special skin–care agents.
3. Sun–protection formulations with UV absorbers being optimally distributed in the horny layer and showing a certain "water resistance".
4. Liposomally encapsulated radical scavengers and related substances of the vitamin E, superoxide dismutase (SOD) or flavonoid type etc.
5. Liposomal formulations of tanning agents such as tyrosine, dihydroxyacetone etc.
6. Fitness frictions.
7. Skin caring after–shaves.
8. Very mild cleansing lotions, which simultaneously provide a skin–care base.
9. Care rinses for the scalp and hair.
10. Treatment of maternal stretch marks.
11. Bath oils.
12. Lotions for use after bath and sauna.

## Lipid–rich systems

Today the most often used formulations are liposomal gels. The demonstration of liposomes in gels can be achieved very precisely by means of the electron microscope (16) (Fig. 13).

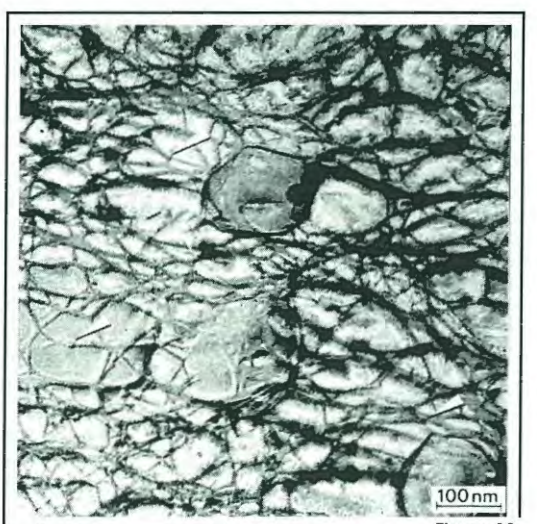


Figure 13

*Electron micrograph of a liposomal skin–care gel after freeze fracture preparation (T. Müller, J. Röding, H. Lautenschläger - 1989)*

The electron micrograpb (Fig. 13) illustrates liposomes in a gel matrix of xanthan gum and aloe vera.
Problems of compatibility with surfactants very often appear, when liposomal dermatologicals

are formulated as creams. Exchange processes take place which are often, in the end, detrimental to the liposomes. These processes become particularly apparent during stress tests at elevated temperature.

For this reason some manufacturers of cosmetics suggest a two-phase treatment for their customers: firstly, application of the liposomal formulation followed by the usual day or night cream. From the point of view of bioavailability of the liposomes this is certainly a good recommendation.

On the other hand, lipid–rich liposomal systems are of particular interest for cosmetics. The capacity of classical liposomes for lipids of the triglyceride type is not sufficient for the amounts required for skin care. When it is remembered that with very few exceptions the concentrations employed for liposomes do not exceed 1% and frequently lie below this.

Some very simple oil–in–water creams with very low proportions of emulsifiers have been described in the literature. Thus, for example, a liposome dispersion can be stirred into a cream base made up of 10% paraffin oil, 0.5% polysorbate, 0.5% sorbitan mono–oleate and 85% water without the liposomal system being destroyed (17).

Instead of using a small amount of emulsifier similar results can be obtained with suitable gel forming agents, such as certain polyacrylates. In these formulations which have a cream–like consistency for oil concentrations of up to 10% it is possible to avoid additional emulsifier completely. The liposomes can be seen distinctly alongside the oil droplets in an electron micrograph of such a formulation (Fig. 14).

A further new possibility for realizing lipid–rich liposomal systems involves specific mixtures of naturally occurring vegetable phospholipids which are able to stabilize oil up to a proportion by weight of 1:1. However, such vesicles no longer take up the typical spherical shape but are partly more reminiscent of a propeller, in whose centre a tiny droplet of oil is to be found (Fig. 15).

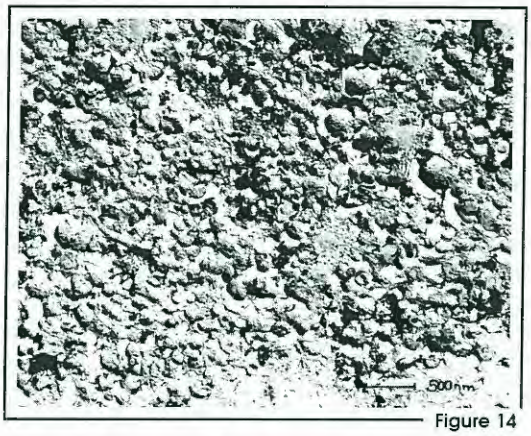


Figure 14

*Electron micrograph of a liposomal gel with 2% liposomes (calculated as a dry substance) and 5% dispersed wheat–germ oil after freeze etching*

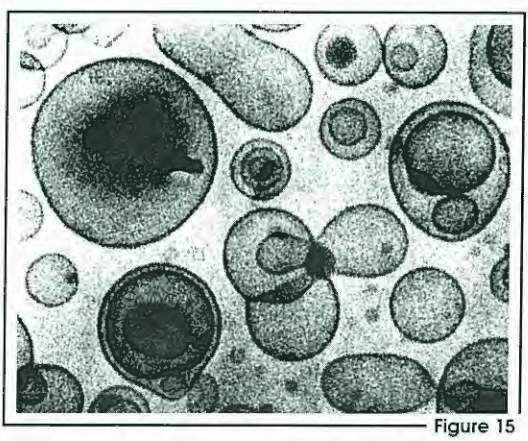

Figure 15

*Cryofixed propeller–liposomes, 1% dispersion (calculated as a dry substance) (18) (J. Röding, 47th Annual Meeting of the EMSA, San Antonio, Texas, USA - 1989)*

Whether this type of liposomes is an ideal base for lipid skin care is still open to question. They are likely, however, to be ideal carriers for lipid–soluble agents. In the ideal case these formulations have the advantage of being able to avoid the use of emulsifying or gel–forming additives, so that the mobility of the vesicles is not restricted.

## Acknowledgements

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