CHAPTER 4: THE USE OF PHEROID™ TECHNOLOGY IN COSMECEUTICAL AND COSMETIC PRODUCTS

4.1 Chapter summary

Pheroid™ is able to penetrate through skin and is able to increase the delivery of active compounds. Results from membrane diffusion studies (they mimic the delivery of active compounds across specific membranes) show significant enhancement in delivery (in some cases as much as 2.5 to 3.5 times) of the active compounds. Pheroid™ vesicles and microsponges are therefore able to transverse most biological barriers, including the skin. The four commercial products based on the related Emzaloid™ technology are in fact topical products and its efficacy has been confirmed by clinical trials. However, the ability to manipulate the system allowed the possibility to use the technology for not only the penetration and delivery of active compounds through the superficial skin layers, but rather for the delivery of active compounds within the epidermal skin layer.

The potential of Pheroid™ technology in the cosmetic industry is described in Chapter 16 of the book: Science and Applications of Skin Delivery Systems (2008), which was written on invitation by the editor; Prof. Johann Wiechers. That chapter is reproduced here as part of this thesis. As the chapter is a reproduction, the numbering and format of the book chapter was kept intact. The chapter also delves into similarities and differences between the Pheroid™ and other oil/water combinations used in the cosmetic and pharmaceutical industries.

Chapter 16

The Design of a Skin-Friendly Carrier for Cosmetic Compounds Using Pheroid Technology

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Abstract

The improvement of delivery from carrier systems used in cosmetic therapies requires attention. A novel delivery system, using Pheroid technology that consists mainly of modified essential fatty acids was evaluated in terms of improved delivery and therapeutic efficacy of topically applied compounds. The investigation included both a theoretical and an experimental study of the structural and functional features of this system, its therapeutic efficacy, its compatibility with the skin and its applicability to the cosmetic industry. The structural study allowed the identification and description of the Pheroid particles dispersed in the continuous phases of the formulations. Various types of Pheroids, each with its own morphology and size, were manufactured. The basic Pheroid has a vesicular structure with sizes ranging from 200–440nm. The membranes of the Pheroids contain pores and a model of the fatty acid packing of the membrane is proposed. The system was found to be versatile with various possibilities for manipulation of the entrapment volumes and efficiencies. In vitro studies include skin diffusion and confocal microscopy studies. In vivo studies include a proof of concept efficacy study on a healthy volunteer and some of the results are shown below. Because of the positive results, the proof of concept study was repeated on a second volunteer with similar results. The Pheroid delivery system was found to enhance the efficacy of various topically administered compounds, as illustrated in both in vitro and in vivo studies. An expanded efficacy trial is now being planned.

Contents of Chapter 16

16.1 Introduction

It is widely recognized that inadequate delivery is the single most important factor delaying optimized application of new classes of molecular therapeutics or cosmeceuticals. Considerable research effort is therefore currently focusing on the continued development of improved delivery systems, which besides enhancing efficacy may also improve safety and overcome tissue-tropism. Although several delivery technologies are available, most of them are hampered to some extent by stability problems, high cost to market and limited field of application, low solubility and bioavailability.[1-3] Pheroid technology, based on what was previously called Emzaloid™ technology, is able to enhance the absorption and/or efficacy of various categories of active ingredients and other compounds and has been shown to result in major improvements in the control of size, charge, and the hydrophilic—lipophilic characteristics of therapies, when compared to other systems.[4-6]

The intellectual property on which Pheroid technology is based was purchased by the North-West University of South Africa in December 2003 from MeyerZall (Pty) Ltd, whose founder, Piet Meyer, had initially developed the technology for the treatment of his own psoriasis. The applicability of this technology in the cosmetic arena lies in the enhancement of the absorption of active ingredients into either the viable epidermis or the underlying dermis. The novelty of Pheroid technology is underlined by patents registered in Europe, the United States, South Africa and China. These patents describe the use of Pheroid technology as a delivery system to promote the absorption and increase the efficacy of dermatological, biological and oral medicines in various pharmacological groups. The effectiveness of Pheroid technology has been illustrated by several national and international clinical trials with products based on this technology.[4-6]

The general perceptions of the permeability of the skin range from the skin as an impermeable barrier to that of a selective sieve. When designing a topical or subcutaneous cosmetic strategy or carrier, the bio-metabolic processes underlying the barrier function of the skin must be taken into account. Although healthy skin minimizes entrance of foreign material and loss of body constituents to the outside, both these processes do occur and the natural absorption process can be exploited in the design of a carrier.[7] The health of the skin can be influenced by vehicles or carriers that will cross the skin barrier or modulate the passage of compounds in either direction.

16.2 Structural Characteristics of Pheroids

The Pheroid delivery system is a colloidal system that contains unique and stable lipid-based submicron- and micron-sized structures, called Pheroids, uniformly distributed in a dispersion medium that may be adapted to the indication. The dispersed structures (dispersed phase) can be manipulated in terms of morphology, structure, size and function. Although the ultra-fine particles that remain suspended in the dispersion medium (continuous phase) in a typical colloidal system are usually between 1–100 nm in diameter, various types of Pheroids are typically formulated to have a diameter of between 200 nm and 2 μm. Parameters such as the required capacity (i.e., the amount and size of the active compound to be entrapped), the rate of delivery and the administration route are taken into account when deciding on the type and diameter of the Pheroids.

The intention in using colloidal systems as carriers of drugs or cosmetic compounds is to enhance the efficacy of the administered compounds while reducing the unwanted side effects.[8,9] The bio-distribution of colloidal particles is dependent on the route of administration and the physicochemical properties of the system, including the particle size and surface

characteristics (e.g., surface charge and surface affinity), as well as the inherent particle characteristics, such as elasticity.[8-10] By manipulating the physicochemical properties, the bio-cosmetic characteristics (e.g., entrapment and release mechanisms) of the carrier systems can be enhanced in addition to overcoming the main physiological barriers described for the skin.

Colloidal systems are typically classified as:

- simple colloids where a clear distinction between the dispersed phase and dispersion medium is found, as in oil-in-water (o/w) or water-in-oil (w/o) emulsions
- multiple colloids in which three phases co-exist—two finely divided dispersed phases, such as in multiple emulsions of w/o/w or o/w/o.
- network colloids that have two phases forming an inter-penetrating network as is found in polymer matrices.

As with these colloidal systems, various types of Pheroid structures can be formulated. However, despite its similarity to such colloidal emulsions, the Pheroid can be formulated into a much greater variety of structures as illustrated in **Figure 16.1**.

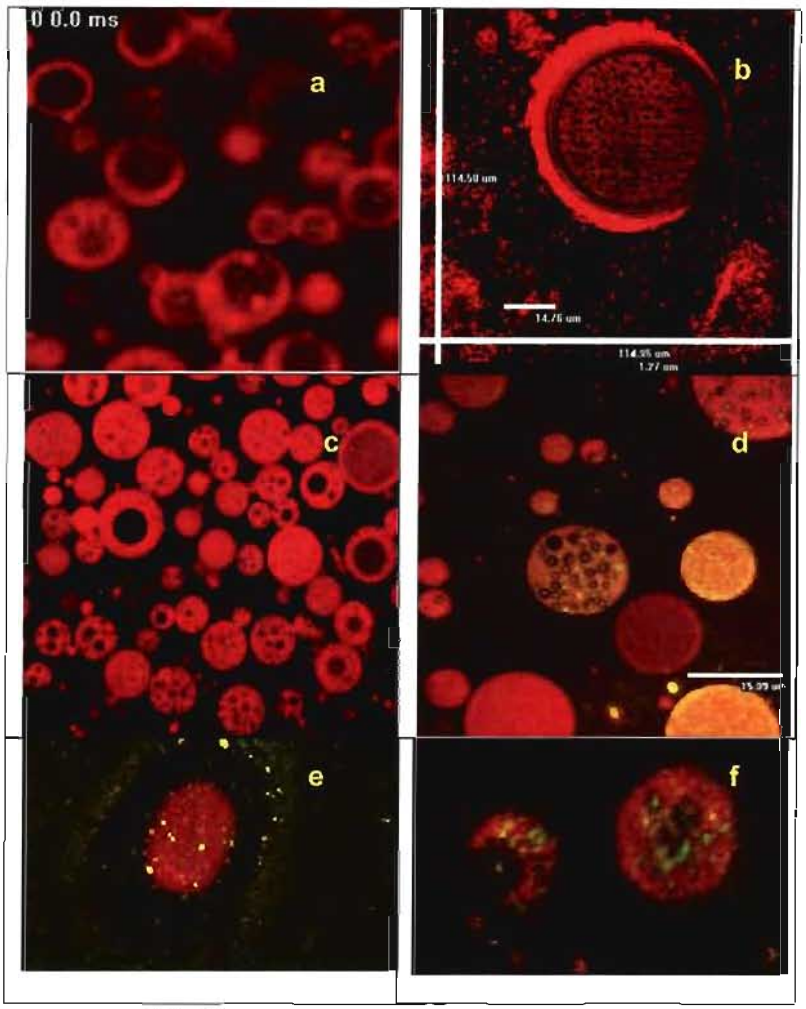

Figure 16.1. *Confocal laser scanning micrographs of: (a) a mixture of liposome-like bilayer vesicles and nanosponges with a mean diameter of 200 nm, (b) a Pheroid microsphere of the reservoir type with a mean diameter of 35–40 µm, (c) colloids with three phases (w/o/w emulsions) with a mean diameter which is submicron, (d) colloids with three phases (w/o/w emulsions) with a mean diameter up to 15 µm, (e) a pH-dependent depot ranging in size from 5–100 µm, (f) Pheroids (red) containing on average 13 auto fluorescent active molecules.*

16.2.1 Ingredients of Pheroids and Molecular Organization of the Pheroid

Pheroids consist primarily of ethylated and pegylated polyunsaturated fatty acids, including the omega-3 and omega-6 fatty acids but excluding arachidonic acid. The fatty acids are in the *cis*-formation and therefore compatible with the orientation of the fatty acids in man. These fatty acids can be formulated with various compounds for novel and innovative dosage forms. Colloidal dosage forms commonly used include liposomes, emulsions and micro-emulsions, polymeric microspheres and macromolecular microspheres.[1,2,11-15] In the design of

the Pheroid, one or more features of each of these dosage forms have been incorporated and it is therefore important to discuss some of these features.

16.2.1.1 Liposomes[1,2]

Liposomes are crystalline spherules typically formed when phospholipids are allowed to swell in aqueous media. They generally consist of concentric lipid bilayers alternating with aqueous compartments; they may be multi-lamellar and therefore quite large (>1 μm). Liposomes can encapsulate water-soluble ingredients in their inner aqueous space and oil-soluble ingredients in the phospholipid membranes without the use of surfactant(s) or other emulsifiers. Water-soluble materials are dissolved in the water in which the phospholipids are hydrated and when the liposomes form, these materials are trapped in their aqueous center. Liposomes have been shown to enhance the penetration of encapsulated active agents into the stratum corneum. By altering the lipid composition their permeability can be changed (e.g., cholesterol may be incorporated in liposomes to confer serum stability) but this has a dramatic effect on the vesicular properties. The addition of gangliosides, sphingomyelin and polyoxyethylene glycol (PEG)-polymerized lipids may increase their circulation or longevity. Exceptional phospholipids can be used to obtain unusual carriers; the use of e.g., phosphatidylethanolamine does not result in bilayers but forms a hexagonal H_{11} phase.

16.2.1.2 Emulsions and Microemulsions or Nano-emulsions[11,12]

An emulsion is a system consisting of two immiscible liquid phases, one of which is dispersed through the other.[11,12] The particles of the dispersed phase are usually between 0.1–10 μm in diameter. Since most drugs are hydrophobic and water-insoluble, o/w emulsions are used as vehicles to deliver such drugs. Nano-emulsions, also referred to as submicron emulsions with droplet diameters in the size range of 0.02–0.2 μm and with a narrow size distribution, can solubilize water-insoluble drugs within the hydrocarbon core. Whether microemulsions should be regarded as true emulsions or as swollen micelles, is a matter of controversy.

Nano-emulsions (o/w or w/o) are generally formed by dispersion or high-energy emulsification methods.[11] It has, however, lately been shown that formation of emulsions by condensation or low-energy emulsification methods is determined by the surfactant phase structure at the inversion point induced by either temperature or composition.[16] Similar studies on nano-emulsion formation, using phase inversion temperature have shown that complete solubilization of the oil in a microemulsion bicontinuous phase is dependent on the minimum droplet size, but independent of the structure of the initial phase equilibria.[16] Nano-emulsions are stable against sedimentation or creaming with Ostwald ripening, due to its small size.[12] According to Solans et al., the main application of nanoemulsions is the preparation of

nanoreactors by the dispersion of nanoparticles using a polymerizable monomer and the use of nano-emulsions as formulations for controlled drug delivery and targeting.[17]

16.2.1.3 Polymeric Microspheres[13]

Polymeric microspheres are carrier systems in which the drug can either be encapsulated by a polymer coat (**reservoir devices**) or entrapped within the polymer network (**matrix devices**). With these diffusion-controlled release systems the drug can be dissolved or dispersed within the device. Biodegradable polymers such as poly(esters), poly(orthoesters) and poly(anhydrides) are common materials used in the production of polymeric microspheres. They are classified according to the hydrolytic reactivity of their chemical linkage, which correlates to some degree with the rate of biodegradation. However, other factors, including the physical properties of the polymer determine the erosion rate of the device.

16.2.1.4 Macromolecular Microspheres[14,15]

In macromolecular microspheres, the drug can either be entrapped within a macromolecular membrane or be bound covalently to the macromolecules, which are soluble in body fluids. Proteins such as albumin, gelatin and polysaccharides such as dextran and cellulose derivatives are the common macromolecules used in these drug delivery systems.

16.2.1.5 Pheroids

In this section, the features common to the Pheroids and the described dosage forms will be highlighted. As is the case with liposomes, Pheroids generally contain a lipid bilayer, but it contains no phospholipids or cholesterol. In contrast to liposomes, Pheroids are formed by a self-assembly process similar to that of low-energy emulsions and micro-emulsions and no lyophilization or hydration of the lipid components is necessary. As in emulsions, Pheroids are dispersed within a dispersion medium, but it contains not only two liquid phases, but also a dispersed gas phase which is associated with the fatty acid dispersed phase. The specific ratio of pegylated to ethylated fatty acids used in the assembling of the Pheroids adds some of the reservoir characteristics of the polymeric microspheres, while the formulation of natural depots (see **Figure 16.1**) is reminiscent of the structure of macromolecular microspheres.

The Pheroid contains one unique component, namely nitrous oxide (N_2O), which is found distributed in association with the dispersed phase throughout the continuous phase. The addition of a dispersed gas phase to the respective oil and water phases thus adds another dimension to the basic Pheroid. The association of N_2O with the dispersed phase has been shown to have at least three functions:

1. Contributing to the miscibility of the fatty acids in the dispersal medium as shown in **Figure 16.2a**.
2. Contributing to the self-assembly process of the Pheroids, as determined by zeta-potential and particle size analysis (personal communication; executed as part of the M.Sc. study of Uys at the North-West University, 2007)
3. Contributing to the stability of the formed Pheroids, as shown by accelerated and formal stability studies, as well as by its stability at high and low pH

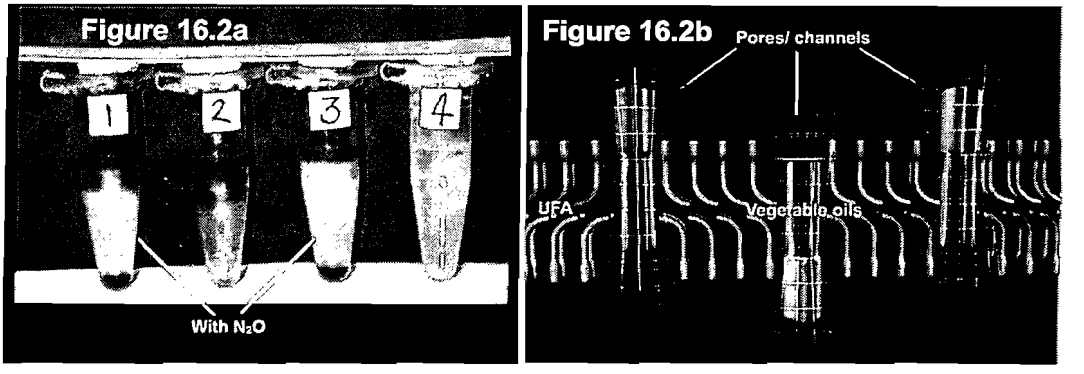

Figure 16.2. *Contribution of N_2O to the miscibility of the oil and water phases (a). A section of the membrane of the Pheroid (b), as calculated by molecular modeling, based on Ab Initio, force field and energy theory as part of a Masters of Science study on Pheroid structure at the North-West University by J. Voges.*

N_2O is a volatile anesthetic compound that is both water- and fat-soluble.[18-20] This characteristic enables the gas to move freely through the epidermal and dermal layers. N_2O has an oil / gas partition coefficient of 1.4, which indicates an average lipid solubility when compared with other volatile anesthetics. The lipid-rich membrane provides an ideal site in which N_2O can concentrate. When sufficient accumulation occurs, the membrane fluidity of specific cells is increased.[18-20] The cellular membranes are dynamic and constantly changing, with not only movement of proteins and lipids laterally on the membrane but with molecules moving into and out of the membrane.[19,20] The increase in fluidity brought on by N_2O and unsaturated fatty acids should increase the movement of hydrophobic molecules or hydrophilic compounds in association with essential fatty acids to move laterally in the membrane to the connecting cells.

Molecular modeling indicates that there is some interaction between the fatty acids and the nitrous oxide, resulting in stable vesicular Pheroid structures. The nitrous oxide essential fatty acid (NOEFA) matrix thus provides a functional model for the transport of hydrophobic and hydrophilic drugs. It was noted in controlled experiments on various formulations that if either

the N_2O or the EFAs were absent from the formulations, the efficacy and stability of the formulation was decreased dramatically.

Empirical evidence of quicker penetration of a given drug such as the anti-inflammatory diclofenac in Pheroids when compared against the drug in another vehicle was obtained in an in vitro study. The data was not obtained in a physiological medium, but evidence of this rapid absorption in vivo was hinted at by the well-known South African rheumatologist Dr. I.F. Anderson in his report after a pilot study where he states: *"A particular impression of the product was its apparent rapid onset of action. This rapid onset has advantages in the treatment of patients and may explain the often reported dramatic improvement" (Clinical Trial Report* to the Medicines Control Council of South Africa and to the sponsor, MeyerZall Laboratories; Patent EP 0 866 713 B1 (2006); Administration media for analgesic, anti-inflammatory and anti-pyretic drugs containing nitrous oxide and pharmaceutical compositions containing such media and drugs).

The size distribution of the vesicles in one of the most used and basic Pheroid formulations with a fatty acid content of 1.89% was determined with the combined use of confocal laser scanning microscopy (CLSM) and membrane filtration. The filtration involved filters of two different pore sizes: 0.22 and 0.45 µm. The distribution is reflected by the chart in **Figure 16.3**.

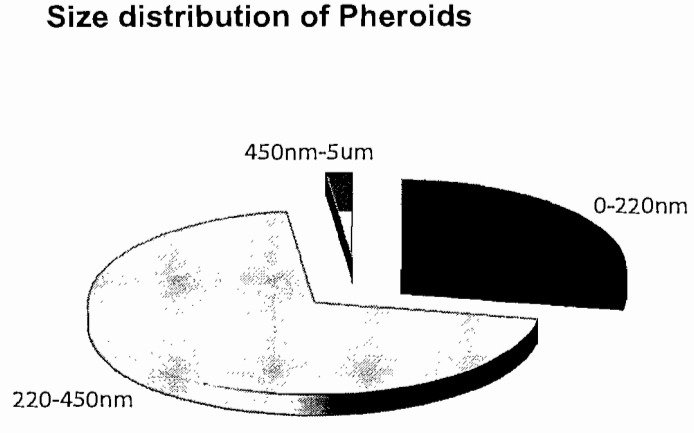

Figure 16.3. *Size distribution of a typical Pheroid formulation prepared through a low-energy manufacturing procedure. Pheroids were labeled with Nile Red and sizes were determined by CLSM.*

The distribution in Figure 16.3 is however slightly skewed, as most vesicles for this formulation measure at an average of 250 nm according to sizing by CLSM. The following factors also influence the size of Pheroids:

- This formulation was not subjected to high-energy procedures such as sonication or extrusion, which result in a decrease in the size of the Pheroids.
- The range and distribution of the sizes of various types of Pheroid are not the same.
- The method of N_2O dispersion influences the average size of the Pheroids. Sparging of the gas through the aqueous phase of the formulation in addition to exposing the formulations to pressure by nitrous oxide decreases the average size of the Pheroids. This decrease in size may relate to mechanical forces, pH–changes due to the gas, which has been found to occur; and the vesicle state, i.e., either gel state or fluid state.

The molecular stacking of the fatty acids in the membranes is extremely specific in two aspects—the sizes of the Pheroids and the pores or channels built into the membranes of the Pheroids. For each type of Pheroid, the sizes formed are pre-determined by the specific fatty acids used, the ratios of the fatty acids used, the saturation state of the fatty acids used, the modification state of the fatty acids, and to a lesser extent by the manufacturing procedure.

For example, transmission electron microscopy (TEM) of Pheroids showed that the sizes of the simplest Pheroid vesicles (containing ethylated linolenic acid, ethylated linoleic acid, and pegylated ricinoleic acid) are multiples of ± 28 nm. For this specific formulation, vesicles with radii of 56.5 nm, 113 nm, 226 nm and 452 nm were detected. One may deduce from the exact doubling that the membrane packing of the fatty acids must be incredibly ordered and that the ingredient with the largest molecular radius (i.e., the pegylated ricinoleic acid) probably determines the packing. The specific packing also relates to the number of pores, which according to modelling must be caused by the pegylated ricinoleic acid. However, it is difficult to prove this except by theoretical modelling. Both TEM and molecular modelling confirmed the existence of pores in the Pheroid membranes at specified distances. **Figure 16.2b** and the insert in the figure show the results of molecular modelling investigations and microscopy of the simplest Pheroid vesicles, respectively.

The design of the Pheroid allows for manipulation of both its structural and functional features (see also the section on "Functional characteristics of Pheroids" below). The surface charge of the Pheroid can be adapted by the degree of hydrogenation of the fatty acids. The mean particle size can be reproducibly manipulated by changing the composition and ratio of the fatty acids. For example, an increase in the pegylated ricinoleic acid leads to smaller

particles with thicker membranes and smaller interior spaces, while hydrogenation of the fatty acids increases the molecular radius of the fatty acids but results in less elasticity of the membranes, but the relationships between ratios and sizes and other characteristics are not simple and direct and are generally determined empirically. The same principle is used in changing the phase behavior of the system. The modifications of the fatty acids are an inherent feature of the Pheroid and not an outer coating of the structure, as is the case in stealth liposomes or pegylation of drugs or delivery structures. The modifications also serve to negate direct uptake of the Pheroid-entrapped compounds by phagocytes, and increase the longevity.

Various investigations into different Pheroid formulations have shown that the structural and functional characteristics of Pheroids can be manipulated by:

- changing the fatty acid composition or concentrations
- the addition of non-fatty acids or phospholipids such as cholesterol
- the addition of cryo-protectants
- the addition of charge-inducing agents
- changing the hydration medium (ionic strength, pH)
- changing the method of preparation (subtle changes may have dramatic results)
- changing the character and the concentration of the active compound
- the addition of sunscreen formulations

All topical formulations based on Pheroid technology currently contain a tocopherol or tocopherol-based molecule, also known as vitamin E or vitamin E derivatives. These molecules are used both as anti-oxidants and as emulsion stabilizers. Vitamin E is a fat-soluble vitamin widely distributed in the membranes of cells throughout the body. The primary function of the vitamin is believed to be the prevention of oxidation of unsaturated fatty acyl residues of membrane lipids.[21] Unlike other anti-oxidant defence mechanisms, vitamin E reacts directly with lipid peroxyl radicals or other oxidants within the cell membrane and is itself converted to oxidation products, thereby breaking the radical chain reaction. Vitamin E is therefore regarded as a scavenger of lipid peroxyl radicals of the chain carrying species that are responsible for propagating lipid peroxidation. Another subsidiary function that has been ascribed to vitamin E is its ability to act as a membrane stabilizer by forming complexes with products of membrane lipid hydrolysis such as free fatty acids and lysophospholipids, thereby counteracting their disruptive effects.[21]

16.3 Functional Characteristics of Pheroids

The efficacy of the delivery of an active compound to skin involves different processes, and depends on the target of delivery—both the tissue as well as the cell type. In the ideal product, each of these processes will be optimized. Delivery of an active compound to the viable epidermis will, for example, involve at least the following processes:

- entrapment of an active compound in the carrier;
- penetration of an entrapped compound across the stratum corneum to the viable epidermis
- uptake of the entrapped compound by the corneocytes
- release of the active compound from the carrier

In an evaluation of these processes, it becomes clear that two different types of control exist: control by the designer/manufacturer of the product and physiological or biochemical control by the cells or tissue targeted. While the product designer/manufacturer cannot control the physiological or biochemical processes, knowledge of these processes and their control may be used to optimize the total delivery process. It is therefore of importance to evaluate the contribution of the carrier or delivery vehicle in these processes.

16.3.1 Pliable System Design and Versatility

As a result of the different target sites in the body for pharmaceutical and cosmetic applications, versatility is one of the requirements of an acceptable carrier system. The structural versatility and pliable design possibilities of the system were discussed under structural features of the Pheroid. The functional versatility is reflected by the enhanced efficacy of a variety of therapeutic compounds, the different routes of administration that have been investigated for Pheroid-based products and the variety of Pheroid-based administration dosage forms that has been used. As this chapter concerns itself with cosmetics, this feature will not be further expanded upon.

Due to the use of a gas as well as the pliable pegylated tails added to the fatty acids, extremely elastic structures are formed. Pheroids are not shattered under moderate pressure or extravascation. The pegylation serves to sterically stabilize the Pheroids and maintain their interior spaces. Furthermore, polyethylene glycol (PEG) has been shown to contribute to increased bioavailability, increased drug stability and extended circulating life, lower toxicity, and enhanced drug solubility.[22,23]

16.3.2 Entrapment Efficiency (EE)

The efficiency of entrapment of a compound can be expressed as the percentage of the initial amount of compound added to the formulation that is entrapped:

$$EE\% = \frac{Amount\ of\ entrapped\ compound}{Total\ amount\ of\ compound\ initially\ used} \quad x \quad 100\%$$

The entrapment efficiency of Pheroid-based products is generally determined by CLSM, and both the Pheroid and the active compound are visualized through fluorescence labeling. An entrapment efficiency of more than 90% has been aimed at for all products in development. Due to the pliable system design of the Pheroid, the number of colloidal particles per volume can be increased or decreased to suit the required concentration of active compound. The interior entrapment volumes of the Pheroids can be determined by the general volume formula (πr^3) where r is the radius of the Pheroids. The theoretical entrapment volume for increasing sizes of vesicular Pheroids can thus be calculated as illustrated in **Figure 16.4**.

Various factors influence internal entrapment volume: the size of the vesicles, the concentration and character of fatty acids, the concentration and character of the active compound, and the hydration medium (ionic strength, electric capacity, presence of charged molecules, etc.). The number of molecules of the active compound that are entrapped within one Pheroid depends mainly on the size, charge and solubility of the active compound. A number of molecules of the active compound can be entrapped within one Pheroid, as illustrated in **Figure 16.1f**, where an average number of 13 compounds (green) were counted per Pheroid. The use of CLSM enables accurate determination of the entrapped compounds by 3-D analysis of optical sections without physical interference. The number of Pheroids required per volume can thus be calculated:

$$Number\ of\ Pheroids = \frac{Total\ number\ of\ active\ molecules}{Average\ number\ of\ active\ molecules\ per\ Pheroid} \quad x \quad 200$$

In this formula the term active molecule can also mean chemical compounds, biological compounds or structures such as peptides, crystals or the smallest unit within which the compound may occur within the formulation. Multiplication of the ratio with 200 is done as the number of Pheroids should preferably be double that of the molecules, to ensure complete entrapment in equilibrium. The simplest Pheroid formulation contains an average of 150

Pheroids/µl or 150,000 Pheroids/ml of formulation. This number can be increased or decreased as required.

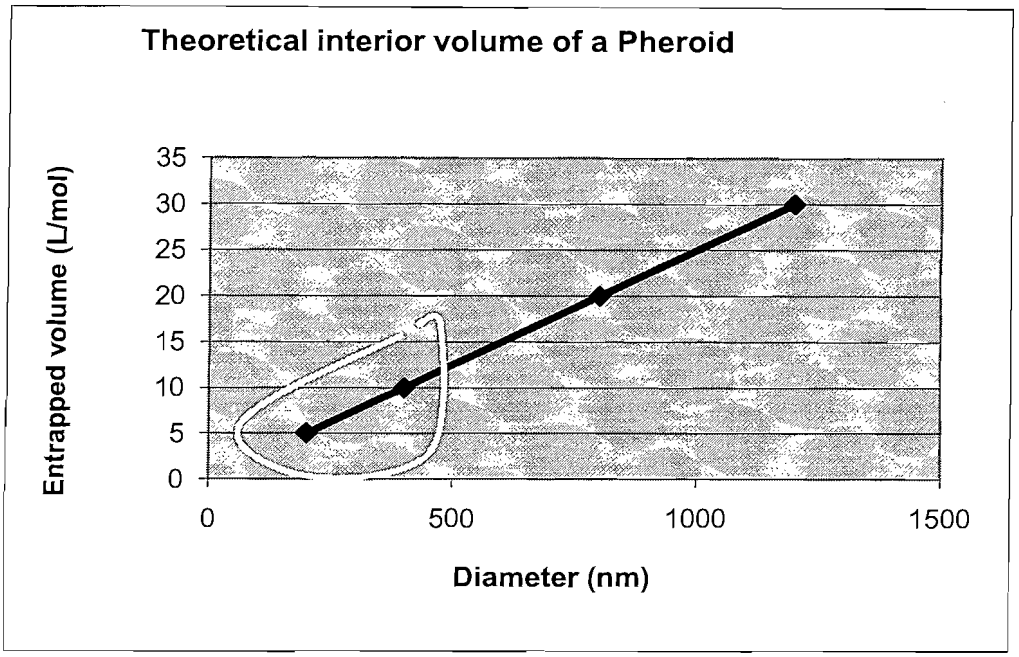

Figure 16.4. *Theoretical interior volume for different sizes of vesicular Pheroids. The white freeform line indicates the size range of the average vesicular Pheroid.*

16.3.3 Penetration Efficiency

The role played by the physicochemical properties of substances, as determinants of skin permeation, has been well documented and will not be discussed here.[24,25] The molecular weight, water solubility, melting point and oil/water partition coefficient are some of the more important physicochemical attributes that influence penetration efficiency and a number of laws, such as Fick's laws of diffusion, describe the role of each of these characteristics.[24] The barrier function of the skin, based on its structure, has also been well described and will not be addressed again, except in for the direct relationship between the structure or function of the Pheroid and that of the skin.[25]

Studies showed that the percentage of active compound delivered to skin was enhanced by entrapment in Pheroid. **Table 16.1** reflects results of membrane diffusion studies, which mimic the transport of active compounds into and across the skin. The compounds named in the Table are used as anti-fungal and anti-viral drugs in the treatment of skin disorders. "PHER" indicates the Pheroid-entrapped product and "COM" a comparable commercial product with the identical concentration of active compound but not Pheroid-entrapped. The amount of compound that was released from the skin was determined by HPLC according to the

applicable protocol as described by the USP. The higher percentage release found for both the antiviral and antifungal compounds indicates that the entrapment of the active compound into Pheroids led to an enhancement in the absorption and transport of both these compounds; with a dramatic increase in the case of the antifungal compound miconazole nitrate.

Table 16.1. Release rates and percentage release per label claim for product tested

Active Agent	Concentration Active (% w/w)	Release Rate ($\mu g/cm^2/h$)	Percentage Release per label claim
Acyclovir PHER	0.5	69.1533	0.1214
Acyclovir COM	0.5	54.0942	0.0952
Miconazole Nitrate PHER	2	389.9238	6.8155
Miconazole Nitrate COM	2	111.2222	1.9466 2

The efficiency of penetration can be measured in a number of ways. The easiest is a comparative investigation, where the enhancement caused by the carrier is determined by comparing it to an existing commercial product. While it is possible to determine the amount of compound by various analytical techniques in different skin layers by skin stripping, CLSM can be used to accurately determine the depth of penetration as well as the amount of active fluorescent compound that penetrated the skin at a specific depth provided the active is fluorescent. In addition, the penetration of the carrier can be determined through pre-labeling with an applicable fluorescent marker.

The enhancement in delivery at different depths in the skin over time can thus be determined and the enhancement factor calculated. This determination leads to different enhancement factors as a function of depth and time. An area under the curve of enhancement factor with time is therefore obtained for each depth. The total enhancement is then calculated from the respective total areas under the curves. In Figure 16.5, the delivery of the active compound is progressively enhanced when compared to the reference product. Through studies of this type, it has repeatedly been confirmed that Pheroids rapidly traverse the stratum corneum with its entrapped compound. One can conclude that the Pheroids—and/or active compounds are within the cells and not located on the outside of the cells. However, it still does not show release of the active compound within the cells for which Phase I or II trials are necessary to conclusively demonstrate that the delivered material is also bioavailable.

16.3.4 Uptake of Pheroids and Entrapped Compounds by Cells

The Pheroid is sterically stabilized by electro-chemical interaction and not by cholesterol, as is the case in most lipid-based delivery systems. Cholesterol results in rigid vesicular structure with an inability to extravasate, i.e., to deform in order to cross densely packed cohesive capillary walls, without fracturing, whereas the electro-chemical stabilization of the Pheroid allows a very elastic vesicular structure. Pheroids have been shown to cross capillary walls. The dense stratum corneum offers a similar challenge and it is thought that the fluidity of the Pheroid membrane contributes to efficient dermal and transdermal delivery.

The uptake of Pheroids by cells may be influenced by the Pheroid formulation and by the mechanism of uptake by the cells. The permeation of the Pheroid formulation is determined by one or more factors:

- the size of the Pheroids
- the morphology of the Pheroids
- the molecular geometry of the fatty acids themselves
- the concentration and ratios of the various fatty acids
- the hydration medium (ionic strength, etc.)
- the pH of the preparation
- the presence of charge-changing molecules
- the presence of molecules that influence the electrostatic milieu
- the character and concentration of the active or drug
- the state of the Pheroid (i.e., either gel state or fluid state or in between)

The mechanism of uptake of the Pheroids by cells is to some extent still speculative, but preliminary evidence suggests that the uptake is actively facilitated by the fatty acid membrane-binding proteins generally present within lipid rafts in the cell membrane. The scope of this chapter does not allow an in depth discussion of this mechanism but the following information is pertinent to Pheroid uptake. The mechanism by which fatty acids are taken up by the cell is a controversial subject and the relative importance of diffusional (passive) versus protein-mediated (active) mechanisms is yet to be determined. However, when presented at high concentrations, free fatty acids can diffuse across biological membranes at rates sufficient to support metabolism. Diffusion rates do not explain the high affinity between cell membranes and Pheroids, or the fast rate of transport of Pheroid-associated compounds across the cell membrane. Neither does it explain the relatively large amounts of compounds delivered by Pheroid-based topical formulations. It is hypothesized (and some evidence exists) that the Pheroids are transported by protein-mediated transfer, i.e., an active rather than a passive

process. Keratinocytes do indeed have a novel fatty acid-binding protein involved in essential fatty acid uptake as well as in recycling free fatty acids from the stratum compactum layer of the epidermis.[26]

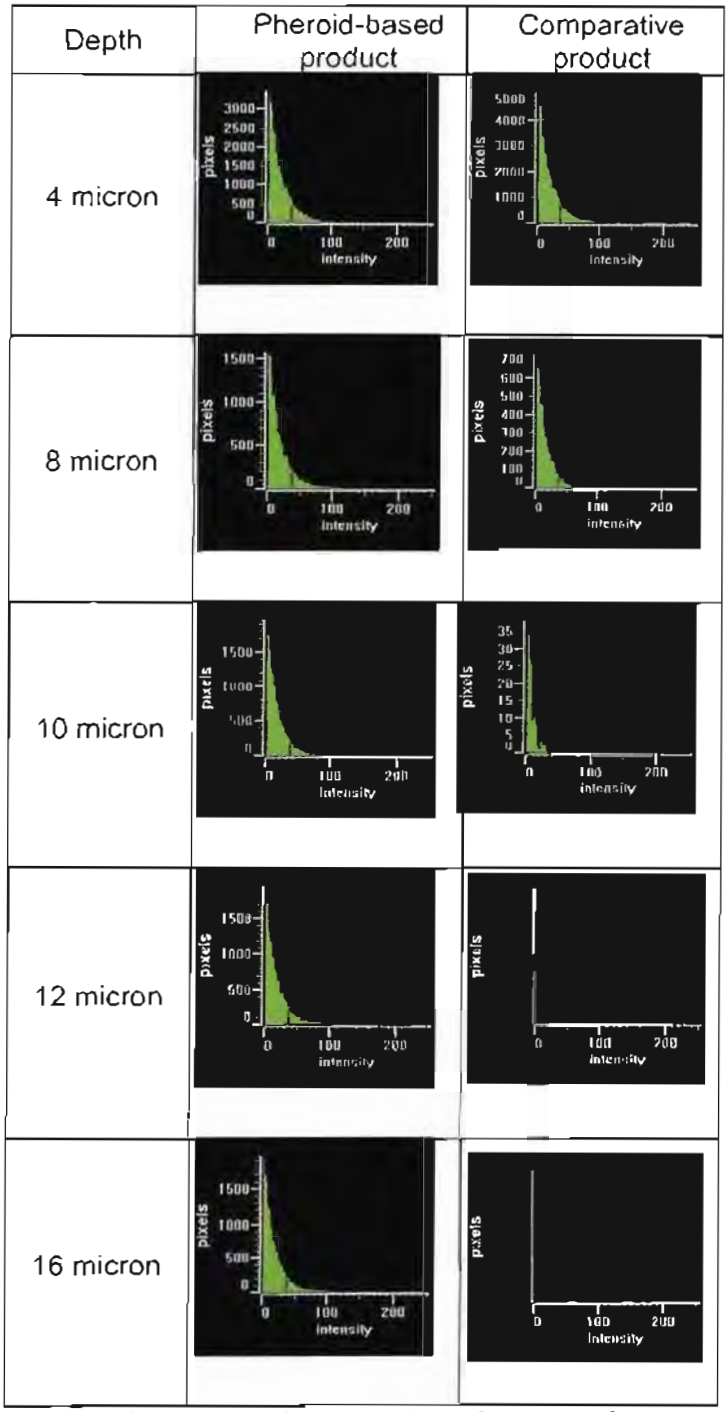

Figure 16.5. *The comparative depth of penetration of an auto-fluorescent active compound (coal tar) into human abdominal skin determined using confocal microscopy in a test and reference product. The Y-axis reflects the intensity of the fluorescence.*

Proteins that efficiently mediate trans-bilayer movement of fatty acids have been identified. These intracellular fatty acid-binding proteins (FABPs) are members of a multigene family, encoding ~15 kDa proteins that bind hydrophobic ligands in a non-covalent, reversible manner.[26] Each of the members of the family so far investigated show unique tissue-specific expression patterns. Furthermore, the members of the family are not equally expressed in all tissues, implying tissue-specific regulation of the various subtypes. This feature may in future be used to specifically target Pheroids to different tissue and cell types. In vivo analyses by molecular and genetic techniques suggest specific function(s) that fatty acid-binding proteins (FABPs) perform with respect to fatty acid uptake, oxidation and overall metabolic homeostasis.[26] The proposed functions of this family include facilitating the influx of fatty acids across the plasma membrane and preventing efflux of these fatty acids, transport of the fatty acids within the cell, thereby determining compartmentalization for metabolism and release of the active compound, the facilitation of solubilization of the fatty acids in an aqueous environment, and modulation of the activity of enzymes involved in fatty acid metabolism and protection of membranes from the possible detergent-like effects of some fatty acids.

16.3.5 Metabolism, Targeting and Distribution

Depending on the type and extent of the fatty acid modifications, the distribution of Pheroids can be influenced. The cellular uptake of the Pheroid is based on various native interactions between fatty acids and cells, amongst these the binding between fatty acids and the FABPs in the cell membrane and the interaction between Pheroids and the lipid rafts present in the cell membrane. Depending on the composition of the Pheroid, they are metabolized in either the mitochondria or the peroxisomes of the cell, resulting in release of the active compound, as confirmed by co-localization studies of Pheroids and various sub-cellular organelles (results not shown).

16.4 Therapeutic Efficacy

Although the efficiency of entrapment and the rate of transport and release of active compounds into and across the skin or the stratum corneum can be indicative of the performance of a delivery system, the proof of the pudding is still in the eating—the effect of a delivery system or carrier should ideally be measured by its contribution to therapeutic efficacy. The formulation of active compounds in Pheroids has been shown to increase the efficacy of a number of such active compounds. Even the effect of essential oils on the skin is enhanced by entrapment in Pheroids.

An example of enhancement of the action of an essential oil, as determined by measuring the hydration state of the skin surface as well as by a surface evaluation of the skin, is shown in **Figure 16.6**. One of the essential oils investigated was *Calendula officinalis* (marigold), which is known for its soothing and cooling qualities and are used in the treatment of dry, damaged and/or sensitive skin. A proof of concept study was performed on a healthy volunteer with pronounced skin sensitivity as well as a dry skin. The use of well-known commercial moisturizers in previous clinical studies led to eruptions, erythema and extreme dryness of the skin of this volunteer. The hydration level of the skin surface was determined with a Corneometer® CM 825[1]. The instrument has been shown to measure the hydration state of the skin reproducibly and accurately to a depth of 10–20 μm. The basic principle upon which the measurement is founded is that of the capacitance of a dielectric medium; a change in the dielectric constant due to a variation in hydration alters the capacitance. The capacitance is detected by a precision measuring capacitor that can reflect very slight changes in the hydration level. The application of products to the skin has a minimal influence on these measurements. The instrument was calibrated and used according to the manufacturer's instructions. The investigations were conducted in a relative humidity and temperature-controlled environment.

The Visioscan® VC 98[2] is an AV-A light video camera with high resolution and has been developed specifically for direct evaluation of the skin surface. The evaluation is expressed as the so-called SELS parameters, an acronym for surface evaluation of live skin. The camera is connected to a computer and dedicated software permits the calculation of a variety of skin surface parameters such as smoothness, roughness, scaliness and wrinkles. Before and after treatment comparisons of the same skin site allow a comparison of trends in skin condition. For the proof of concept study, all determinations were done in triplicate and non-continuous scans were taken to calculate the SELS parameters. The standard error was found to be very small. The threshold was calculated individually for each micrograph/determination. One of the characteristics of dry skin that was determined was scaliness—the smaller the value of scaliness, the more hydrated is the stratum corneum (comparable to roughness, but scaliness is more extreme roughness).

[1] Corneometer CM 825, Courage & Khazaka, Cologne, Germany
[2] Visioscan VC 98, Courage & Khazaka, Cologne, Germany.

Fig 6a: Hydration status of skin

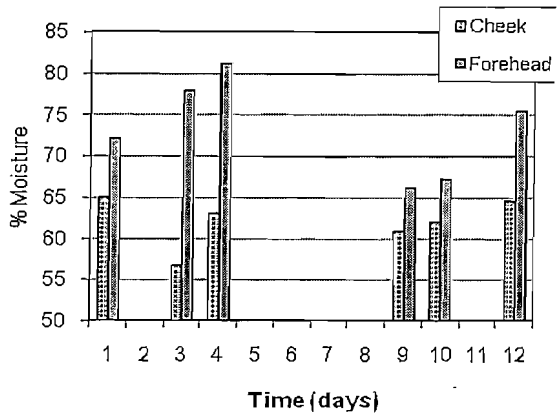

Figure 6b: Scaliness

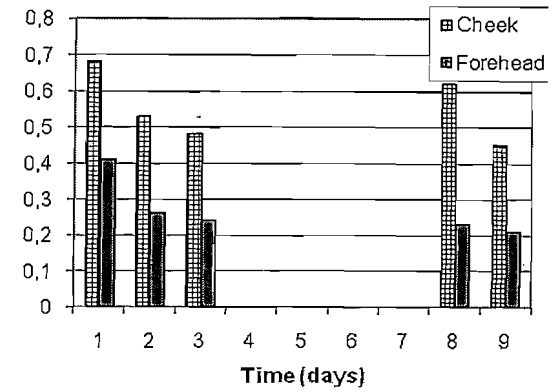

Fig 6c: Wrinkles

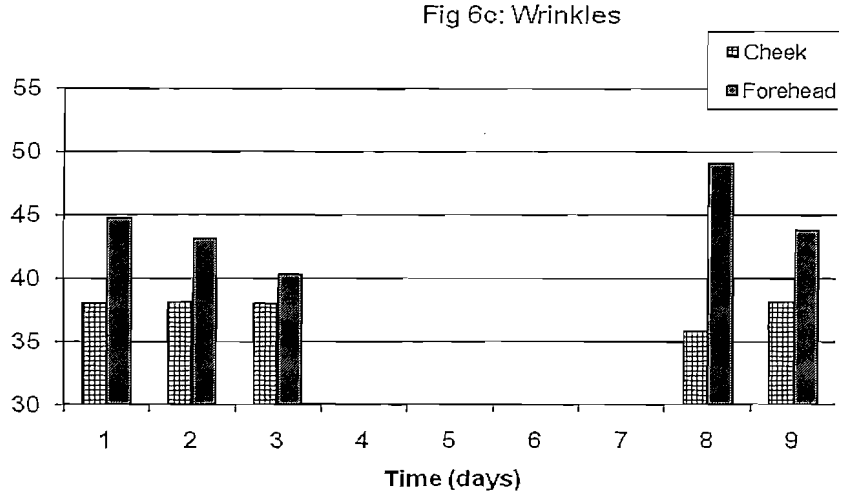

Figure 16.6 *illustrates some of the parameters determined in the described proof of concept study. The baseline Pheroid product (reference product) and essential oil product were identical except for the addition of 0.1% (v/v) Calendula officinalis to the Pheroids in the test product. The baseline Pheroid product was applied to the face and hands of the individual for a week before the evaluations started in order to exclude any sensitivity reaction and to allow determination of the contribution of Pheroids to the hydration status (Figure 4a), the scaliness (Figure 4b) and the surface wrinkles (Figure 4c) of the skin. The baseline hydration of the skin was determined on Day 7 and used as the value portrayed in the graph for Day 0. The test product (i.e., Calendula officinalis-containing Pheroid formulation) was then applied in similar quantities, to the same areas for four days, with hydration measurements as indicated. Days 2 and 5–8 were wash-out periods with no application of any moisturizer. Analysis of this result suggests that the wash-out period should probably have been longer. Nevertheless, the decrease in hydration during the wash-out period indicates that the Pheroid itself has a significant hydrating effect.*

Scaliness (Figure 4b) was similarly determined, but the baseline is portrayed in the graph as Day 1. No change in wrinkles (Figure 4c) was observed for the cheek, but a definitive decreasing trend in wrinkling was observed on the forehead, especially after the wash-out period. For both the cheek and forehead areas, treatment with the Pheroid-entrapped essential oil showed a fast response and therapeutic efficacy in terms of the parameters investigated.

The improved efficacy was also found for pharmaceutical compounds that can be applied topically, as shown by disk diffusion or zone of inhibition studies (**Table 16.2**), in which the efficacy of various anti-infective agents against a number of micro-organisms was tested. The larger the area (as measured in millimeters) of inhibition in the growth of the specified micro-organisms, the more effective the product is against the micro-organisms.

In every single case, the zone of inhibition was enhanced by formulations of the active compound contained in Pheroids. All the above formulations made use of one single Pheroid type. Reformulation of ciprofloxacin and erythromycin in microsponges has since increased efficacy. As the above analysis does not reflect a possible increase in bioavailability, enhanced bioavailability should show a further increase in the efficacy of the Pheroid formulations.

Table 16.2. Zone of Inhibition study: Five commercial anti-infective products against Pheroid-formulations of the same active compound.

Active Agent	PHER / COM	Dose mg / 5ml	S. Aureus	P. Aerugin	B. Cereus	E. Coli	A. Niger	C. Albicans
Cloxacillin	PHER	125	30.74	23.96				
Cloxacillin	COM	125	29.45	19.78				
Erythromycin	PHER	250	26.7		29.89			
Erythromycin	COM	250	25.84		27.78			
Ciprofloxacin	PHER	250	33.05			35.78		
Ciprofloxacin	COM	250	30.14			33.4		
Cotrimoxazole	PHER	240	13.95			24.64		
Cotrimoxazole	COM	240	11			22.83		
Itraconazole	PHER	50					16.03	14.28
Itraconazole	COM	50					11.47	10.21
Control			9	9	9	9	9	9

Enhancement of the therapeutic activity or an increase in the therapeutic index (TI) of the actives by the Pheroids has thus been established. The enhancement is thought to be a result of:

- deposition of compounds within organisms by the Pheroids
- enhancer effect of individual fatty acids and N_2O upon barrier permeability and fluidity
- lipid concentration — the higher the free fatty acid concentration, the higher the delivery up to saturation point
- increased thermodynamic activity of active compounds due to less favorable environment for charged molecules
- increased partitioning into cells because of ion-pairing effects in a low dielectric medium and binding to FABPs in membranes

While the delivery system is not limited to topical application, research has indicated that many compounds, including peptides, may be administered topically instead of orally, thus by-passing possible product degradation, gastro-intestinal side effects and first-pass clearance by the hepatic system.

16.5 Compatibility between the Skin and Pheroid Formulations

The hypothesis that the efficacy of transdermal and cosmetic therapies is enhanced by the Pheroid delivery system was tested both in vitro and in vivo in studies similar to that described above. However, enhancing therapeutic efficacy may be accompanied by harsh side effects. Therefore, the design of a skin-friendly carrier needs to be based on the inherent requirements of the skin. The nature and concentration of the therapeutic compound, the vehicle in which this compound is presented, as well as the condition of the skin will ultimately determine its therapeutic efficacy.

The skin is a dynamic structure under homeostatic control. The dynamics of the formation of the skin barrier is directly relevant to the etiology of pathological skin conditions and to the development of strategies designed to treat such conditions.[24] Cornification and desquamation are intimately linked; the synthesis of the stratum corneum occurs at the same rate as its loss in normal skin and under normal conditions. A cosmetic strategy should therefore be aimed at maintaining this homeostasis, and the design *and the content* of carrier vehicles may be dedicated to a specific cosmetic indication.

16.5.1 Inherent Therapeutic Effect of the Essential Fatty Acids (EFAs) of Pheroids

The intercellular regions between the organelle- and cytoplasm-poor corneocytes are composed mainly of lipids, generated by exocytosis of lamellar bodies during terminal differentiation of the keratinocytes to corneocytes. The intercellular lipid forms the only continuous domain in the stratum corneum and is required for a healthy skin and competent skin barrier.[27,28] This continuous domain is crucial in the application of any topical formulation; the disturbance of these lipids with compounds such as non-polar solvents is guaranteed to reduce the barrier function with a resultant increase in trans-epidermal water loss (TEWL) and dehydration of the skin itself. It is strongly suggested that such compounds should be avoided in any cosmetic preparation.

The intercellular lipids are produced by lamellar bodies in the underlying viable epidermis and consist of phospholipids, free sterols, cholesterol sulfate, acylceramides and acylglucoceramides.[24-28] The various factors that have a negative impact on the barrier include ageing, exposure to different climactic conditions and certain chemicals, pathological conditions and diet (see below). The repair of the skin condition and recovery of the skin barrier is a multi-step procedure that involves the synthesis of the primary precursors of the intercellular lipids, which is, in turn, dependent on the ratios of the available fatty acids.[29] In psoriasis, for instance, the covalently bound lipid is similar to normal skin, but the level of ceramide 2 is reduced and the level of free fatty acids and omega-hydroxy acids are correspondingly increased.[30] During maturation from basal epidermal cells to corneocytes, a progressive increase of especially the fatty acid content, the ceramide content, and the cholesterol content of cells has been observed, whereas nearly no phospholipids are present in the corneocytes. In addition, the proportion of long chain fatty acids in the stratum corneum is much higher.

The lipid synthesis in keratinocytes is independent of the nutritional status of the individual, with the single exception of the two essential fatty acids (EFAs), linoleic and linolenic acid, which must be obtained from nutritional sources (i.e., through the blood and vasculature) and which are essential in the formation of the skin barrier.[31,32] The fatty acid metabolism of keratinocytes seems to be unique and not characteristic of most other mammalian membranes. Exogenous application of oleic acid, for instance, has been shown to alter the much more rigid skin barrier, whereas the more flexible intestinal epithelium barrier is not influenced by the presence or addition of unsaturated fatty acids. The epidermal metabolic conversion rate of the EFAs is high, with the most abundant EFA being the 18-carbon n-9 n-12 fatty acid, linoleic acid (LA; C18:2)). Linolenic acid (9,12,15-octadecatrienoic acid; C18:3) plays a direct role in restoring the barrier function of the stratum corneum and influences epidermal permeability to

normalize the hydration of the skin. This normalization of hydration is necessary in the treatment of dry skin and the itchiness of atopic skin. Deficiencies of EFAs result in a characteristic dry scaly skin disorder, ageing of the skin and excessive epidermal water loss, with associated loss of the barrier function of the skin.[33]

In an effort to design a skin friendly carrier, the main fatty acids used in the Pheroid are the EFAs, although slightly modified. Fatty acids are the major source of energy in most mammalian cells and comprise an integral structural component of all cell membranes.[34] EFAs, i.e., linolenic acid and linoleic (LA) acid (but excluding arachidonic acid), are inherent components of Pheroids and are primary constituents in all formulations. Depending on a particular indication and the pathology thereof, the unsaturated fatty acids are supported by additional long chain unsaturated fatty acids, such as all *cis*-5,8,11,14,17-eicosapentaenoic acid (EPA; C20:5), all *cis*-4,7,10,13,16,19-docosahexaenoic acid (DHA; C22;6) and plant fatty acids or sterols/sterolins, such as ricinoleic acid (12-hydroxy-9-octadecenoic acid). The spatial orientations of oleic acid (18:1n-6), linoleic acid (18:2n-6), and alpha-linolenic acid (18:3n-3) are increasingly more extended and result in higher membrane fluidity. In contrast, carriers containing saturated fatty acids, such as stearic acid, cause relatively solid domains in the membrane.[35]

During active processes in the epidermis, the EFAs are transformed into (mainly) monohydroxy fatty acids, which have been shown to exhibit anti-inflammatory properties.[36] In vitro studies with LA and the epidermal fatty acid enzyme 15-lipoxygenase (EC 1.13.11.33), showed that the epidermis is also unique in that it preferentially metabolizes LA to hydroxyoctadecadienoic acid (13-HODE), which helps to maintain the homeostasis between desquamation and proliferation in the epidermis. Ziboh et al. showed that the 13-HODE–metabolite has a selective effect on total epidermal protein kinase C (PKC) activity and that the two epidermal PKC isozymes are selectively and differentially expressed.[36] Their results suggest that the amount of LA present in the epidermis can, as precursor of 13-HODE, modulate epidermal PKC activity and expression, which are purportedly associated with epidermal hyper-proliferation. It was found that in animals deficient in EFAs, a reduction in 13-HODE paralleled both epidermal hyper-proliferation (scaly lesions) and elevated expression and activities of the PKC-isozymes when compared with control animals. The deficiency was reversible and resulted in the selective regulation of PKC-beta expression as well as suppression of epidermal hyper-proliferation. Thus, it seems that the epidermal concentration of 13-HODE, which is normally obtained from dietary LA, but may be obtained from the Pheroids, plays a role in in vivo modulation of cutaneous cell proliferation. Although the 18-carbon 13-HODE derived from LA exerts moderate anti-inflammatory effects in vitro, it exerts potent anti-proliferative effects in vivo, as evidenced by the reduction of epidermal scaly lesions. The 15-

lipoxygenase mono-hydroxylated metabolites generated from EFAs in the skin may, in concert with other cellular processes, attenuate inflammatory and proliferative cutaneous disorders.[36]

The skin lacks the elongation enzymes necessary to synthesize arachidonic acid (C20:4) from LA (C18:2). This lack means that the supplementation of topical actives with the appropriate essential fatty acids may generate local cutaneous anti-inflammatory and anti-carcinogenic metabolites, which, in turn, could result in a reduction of transepidermal water loss, skin aging, inflammation and excessive cell division, and therefore be of use in the treatment of minor and major skin disorders. The end products of the various enzymatic reactions of the EFAs are prostaglandins and other eicosanoids. Supply of C18:2 n-6 (LA) will predominantly result in the production of PGE_1, a strong anti-inflammatory in itself. Likewise the supply of C18:3 n-3 (alpha-linolenic acid) will result in the production of PGE_3, also an anti-inflammatory agent.

Dihomo-linolenic acid (DGLA; 20:3n-6), although a small constituent of normal epidermis, is formed as an elongation product of linolenic acid (18:3n-6), one of the components of Pheroids. When its concentration is elevated in the tissue, it is metabolized by epidermal cyclooxygenase (COX) to prostaglandin of the 1-series (PGE_1) and also by 15-lipoxygenase to 15-hydroxyeicosatrienoic acid (15-HETrE).[37] Human epidermis contains an active elongase enzyme that converts the linolenic acid to DGLA. DGLA has a higher specificity for COX than arachidonic acid (AA), whereas eicosapentaenoic acid has a higher specificity for lipoxygenase than AA.[37] This combination effectively blocks the production of pro-inflammatory PGE_2 and leukotriene LTB_4 from AA. Supplementation of diets with evening primrose or borage oil raises epidermal PGE_1 and 15-HETrE concentrations. Because the applicable desaturase enzyme is deficient in the epidermis, alpha linolenic acid is not metabolized in significant amounts to arachidonic acid. The reported beneficial effects of evening primrose oil in the clinical management of inflammatory hyper-proliferative disorders of the skin may be due, at least in part, to epidermal generation of PGE_1 and 15-HETrE from elevated tissue DGLA concentrations. It is our contention that the linolenic acid in the Pheroids functions in a similar manner after topical administration.

The composition of Pheroids is designed to support the binding of the EFAs to the FABPs of the keratinocyte, thereby enhancing cellular uptake of therapeutic compounds entrapped in the Pheroid. The maintenance of the intercellular lipid layer that provides the barrier properties of the skin is at the same time supported and transepidermal water loss inhibited by the essential fatty acid component of Pheroid.

16.6 Possible Applications of Pheroid Technology in Cosmetics

Proliferation involves mitosis of the stem and transit-amplifying cells which are primarily situated in the stratum basale of the epidermis. Proliferation of these cells can be directly influenced by diffusion of soluble factors from the underlying dermis or by transport of topically applied molecules through the cohesive stratum corneum. Although the cells present in the supra-basal layers are programmed for terminal differentiation, they have been shown to retain a capacity to respond to external stimuli such as transforming growth factor beta and retinoids.[38] Retinoids have been formulated with Pheroids and are specifically used for the treatment of skin eruption and enhancement of healthy and glowing skin. Other molecules that have an influence on the proliferation and response pathways, and that can be used with success in the Pheroid carrier, are vitamin D, hydrocortisone, epinephrine, extracellular adhesion and signaling molecules and calcium.

The efficacy of a number of anti-infective agents used in topical applications was shown to be enhanced by entrapment in Pheroids in either in vitro or in vivo studies. These include gentamycin, sulphamethoxazole, trimethoprim, cloxacillin, acyclovir, ciprofloxacin, miconazole nitrate, and neomycin. Some drugs, such as proteins or peptides, may induce an immunological response or adverse intolerance reaction. Masking of the compounds by Pheroids may reduce recognition by the patient's immune response. Frequency of dosing can be reduced without diminishing potency, or higher doses can be given to enhance therapeutic impact. The Pheroid system has the potential to minimize cytotoxicity, such as that symptomized by the membrane damage caused by active compounds such as miconazole nitrate.

The following compounds have been successfully formulated with Pheroids: alpha and beta-hydroxy acids, including fruit extract and salicylic acid, various types of collagen, hydrolyzed elastin, vitamin C, anti-oxidants and applicable preservatives, Phytotal V7, Konakian amp and a number of essential oils.

16.7 Conclusions

The vast majority of delivery systems can structurally be characterized as colloids. The surface properties and interfacial interactions of such colloids with the biological milieu determine the therapeutic profiles of the delivered compounds to a large extent. An understanding of the colloid and interfacial perspective of a delivery event enables one to manipulate the colloidal systems.

Kostarelos summarized the biological features that can be engineered by manipulation of the colloid and interface characteristics of delivery systems.[39] These features include surface charge, phase behavior, steric stabilization, endosomal escape and mean particle size. Some of the aspects not addressed by Kostarelos are the stability of the system, the mechanism of cellular uptake, the mechanism of release of the entrapped molecule or compound, the metabolism of the delivery system, the body distribution of the delivery system and the clearance of the components of the carrier from the body. The delivery of therapeutics requires definition of all the stages involved in a delivery event and determination of all critical parameters.

The composition of fatty acids in membranes has a significant effect on membrane fluidity and in functions such as transport and signal transduction. Fatty acids are also precursors for various lipid signaling molecules produced via the cyclooxygenase, lipoxygenase and epoxygenase pathways. The resultant lipid second messengers exert their effect through both cell-surface and intracellular receptors, illustrating the importance of the composition of fatty acids in topical formulations.

Essential fatty acids contained in the Pheroid system are thought to play a dual role. Firstly, the fatty acids are the primary component of the delivery system. Being EFAs, they are inherent components of the skin, and should be recognized as such by the immune system with no immunological implications. Secondly, the presence of the selected fatty acids probably contributes to the normalization of the physiological micro-environment, resulting in added anti-inflammatory action by the EFAs, suppression of epidermal hyper-proliferation, normalizing the water barrier of the skin, and fast and efficient delivery due to binding to the FABPs situated in the membranes of the cells.

The Pheroid is probably one of the most effective, versatile and inexpensive delivery systems in commercial use. All components used in the manufacturing of Pheroids are pharmaceutically safe and the system is based on the naturally occurring molecules of the body. The stability of the Pheroid delivery system has been proven for Pheroid-based commercialized products. Nevertheless, although various studies support the use of Pheroid technology in cosmetic and pharmaceutical applications, the elucidation of all critical parameters is necessary if the technology is to be used to its full potential.

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