CHAPTER 8

Oleic Acid-induced Skin Penetration Effects of a Lamellar Delivery System

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ABSTRACT: *The structure and thermodynamic state of the skin's lipid barrier are correlated with the skin's penetration profile in this in vitro study using a delivery system composed of phospholipids, oleic acid, oil and water. Elevated percentages of oleic acid in the system affect the lipid barrier's structure and thermodynamic properties.*

When designing a topical delivery system, it is important to understand the possible penetration pathways. There are principally four routes for skin penetration (see **Skin Penetration Routes**). Of those, the intercellular pathway through the intercellular lipids is the route for most molecules. Water also penetrates via this pathway.

The organization of the intercellular lipid domains in the stratum corneum (SC) is considered to be very important for the skin barrier function. The SC lipids are composed mainly of long chain ceramides, free fatty acids and cholesterol. These form a bilayer lamellar structure that, although organized, shows a multifaceted polymorphism. The intercellular lipids are predominantly present in their gel crystalline form, but with a subpopulation of lipids in their liquid crystalline form. The solid gel phases are mainly composed of saturated lipids, whereas the liquid crystalline phases are mainly composed of unsaturated lipids.

Skin Penetration Routes

There are four major known ways for skin penetration.[17]

- Intercellular pathway, between the cells, through the intercellular lipids. Used by most molecules and water. The volume of this area is approximately 5–30% of the total volume of the stratum corneum.
- Transcellular pathway, through the keratinized corneocytes
- Pilosebaceic pathway—penetration through hair follicles. Mainly for very lipophilic molecules, but also for molecules in combination with certain surfactants and glycols. Although the available area for diffusion is approximately 1% of the total skin area, it can be the path of choice when penetration to the follicles is required.
- Polar pores pathway consists of aqueous "islands" that are present between cells and are surrounded by polar lipids.

This article describes physical characterization and in vitro skin penetration of a delivery system composed of lecithins, oleic acid, oil and water. A skin penetration study reported here suggests a correlation between the structure and thermodynamic state of the system and its skin penetration profile. While small soft vesicles containing 5% oleic acid allowed penetration to deep layers of the skin, lamellar sheets generated with 15% oleic acid showed a creation of a reservoir in the SC.

This technology is a novel approach to delivering compounds to skin subtissues. It can be tailored to allow the creation of a reservoir in either the SC or the live epidermis and dermis.

The Lipid Barrier

Human skin serves as an efficient barrier for the penetration of compounds. Its upper layer, the SC, has a unique structure in which the dead corneocytes are cemented by intercellular lipids and glycoprotein structures (desmosomes) that are covalently bound to the cell membrane. These intercellular lipids are organized in a lamellar structure.

One class of lipids is lecithins—the most common phospholipids and a major component of all biological membranes. Lecithin can be separated from either egg yolk or soy bean. Chemically, lecithin is phosphatidyl choline. Commercially, it refers to a mixture of neutral and polar lipids. Lecithin contains the ammonium salt of choline connected to a phosphate group by an ester linkage. The nitrogen has a positive charge, as does the ammonium ion. The head of a phospholipid is hydrophilic; the tail is lipophilic.

In biological systems, phospholipids form bilayers, in which the lipophilic tails line up against one another, forming a sheet-like lamellar membrane with hydrophilic heads on both sides facing the water. Under certain physiological conditions, this membrane can spontaneously form globular liposomes, or small lipid vesicles that can then be used to deliver proteins and other materials into living cells. Some of those conditions are described next.

Thermodynamic factors: The primary thermodynamic factor favoring the formation of lipid bilayers in an aqueous environment is the "hydrophobic effect." The bilayer structure favors the interaction of the phospholipids' polar head groups with the aqueous environment, while the lipophilic tails, shielded from contact with water, interact with one another. This unique arrangement allows for relatively high freedom of movement, therefore the resulting vesicles are essentially unstable and may exist in equilibrium with other systems that possess a similar structure, such as micelles or lamellar sheets. The main interaction between lipids in a membrane structure is, by nature, hydrophobic. Only very few lipids in a membrane will remain in their monomer form.

The ability of membrane components to move within the lamellar structure is determined by the membrane's state: temperature, pressure and level of saturation. These three factors are the key factors affecting lamellar condition. The organization of phospholipids in membranes is known to exist in two major thermodynamic states: gel and liquid crystalline. The transformation between the two states is termed *mesomorphism*. In general, low temperature and high pressure will yield a gel state, whereas high temperature and low pressure will enhance the formation of a liquid crystalline state.

In a liquid crystalline state, the lipophilic chains are randomly oriented and fluid. The gel state presents a more restricted lipid-chain motion. In this state, the lipophilic chains are fully extended and closely packed, they are ordered and their motion is highly constrained.[1]

While in their liquid crystalline state, lipids in the membrane can undergo rotational and lateral movement. The lipid chain can bend, tilt and rotate in place. These are very rapid movements with a constant rate of 10^{-9} sec (a billion times every second). Lipids in the membrane will most likely tend to migrate (diffuse) in the bilayer itself (lateral movement) and are restricted in their movement between the two bilayers of the membrane (transverse diffusion). Lateral diffusion will occur approximately one million times per second. If transverse movement does happen, its rate will be relatively slow and will occur once every 100,000 sec.

Upon reaching a certain energy level, phospholipids in the membranes will transform from a gel state to a liquid crystalline state. The temperature at which this transition occurs is known as the transition temperature (Tm). The Tm is characteristic of the lipid composition in a given membrane. Only highly purified lipid systems will provide sharp, well-defined Tm and in most cases this transition will be reversible.

Effect of cholesterol and fatty acids: The thermodynamic properties of a given membrane will be highly dependent on its composition. It is known, for example, that the addition of cholesterol to phospholipid membranes can affect its transition temperature.[2] Due to its "flat" structure and relatively small size, cholesterol fills in the gaps created by imperfect packing and modulates membrane fluidity. It acts as a Tm "buffer" that broadens the transition temperature range, and its activity is temperature dependent.

At temperatures below the Tm, when the membrane exists in a gel-organized state, cholesterol has been reported to prevent the ordered packing of the acyl chains, and to increase their freedom of motion. At temperatures greater than the Tm, the rigid ring of cholesterol reduces the freedom and fluidity.

Fatty acids tend to move spontaneously—a so-called "flip-flop movement"—across the bilayer of vesicles. Their rate of movement across the bilayer may affect their mechanism of transport across the membrane. Fatty acids can be present in membranes in their ionized or non-ionized form. The non-ionized form is more likely to be integrated into the lamellar structure and move within the lamellar structure. This "flip-flop" movement will typically occur across the phospholipid bilayer. The pH of the system can therefore play a significant role in its structure and fluidity.[3]

Increasing the levels of unsaturation in the fatty acid may lower the Tm. Introducing a double bond into the lipid acyl group induces a curvature in the chain that requires lower energy, and thus leads to a non-ordered structure packing.[4]

A different effect was observed when a saturated fatty acid was introduced into a dipalmitoylcholine (DPC) bilayer. The addition of palmitic acid (PA) increased the bilayer Tm, and this increase depended on the PA concentration. In addition, DPC molecules in their gel phase became more rigid when PA was added. Other saturated fatty acids, such as stearic acid or myristic acid, demonstrated similar effects.[5]

Interestingly, the presence of certain fatty acids, including oleic acid, can lead to the transformation of the lamellar structure to a nonlamellar state termed hexagonal II phase.[6] This is a three-dimensional structure with long tube-like lipid structures surrounding a water compartment. Some phospholipids will tend to form this structure as a result of reduction in water content.

A Skin Penetration Study

How do structural changes in the skin lipid barrier affect the barrier's ability to prevent the penetration of compounds? In the remainder of this chapter, that question is addressed in terms of the physical characterization and in vitro skin penetration of a delivery system that is composed of lecithins, oleic acid, oil and water. Transmission electron microscopy (TEM)and differential scanning calorimetry analysis are used to evaluate the effect of elevated percentages of oleic acid on the system's structure and thermodynamic properties.

If the thermodynamics of skin lamellae is altered from organized to fluid state, a higher skin penetration may be achieved. Here, the authors attempt to develop a lamellar delivery system that is flexible and fluid in nature. The rationale is that when applied to the skin, such a system will fuse into the intercellular skin lipids and temporarily trigger the skin lipids' fluidity to allow higher partitioning of entrapped compounds into it. Starting with simple compositions, an elevated percentage of oleic acid was incorporated into the system and physical properties were determined. In order to understand the correlation between a system's composition, its properties and interaction with the skin, an in vitro study was designed to follow penetration of a marker into and through the skin.

Method and Procedures

Preparation of samples: Mixtures of soy lecithin, oleic acid, and natural oils such as jojoba or rice bran oils were prepared by cold processing. The oil phase was then mixed with water in various ratios, resulting in three formulations containing oleic acid at 0–15% w/w, oil at 15–20% w/w and soy lecithin at 10–20% w/w. Stabilization of the systems was achieved by adjusting hydrophilic lipophilic balance (HLB) using different types of lecithins.

Transmission electron microscopy: Preparations were observed using TEM after negative staining. A drop of diluted preparation was mounted onto a copper grid. Phosphotungstic acid (PTA) or uranyl acetate (UA) negative stain was then applied, followed by drying for a