

IN ORDER TO  
STOP LOOSING  
EFFICIENCY OF  
YOUR SKIN CARE,  
PICK OUT  
A SKIN CARE  
WITH GPS.\*



GUIDING  
AT SURFACE



GUIDING  
MEDIUM



GUIDING  
IN DEPTH

# SCIENTIFIC FILE OF INGREDIENTS: GPS-S, M & P

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# SYNOPSIS

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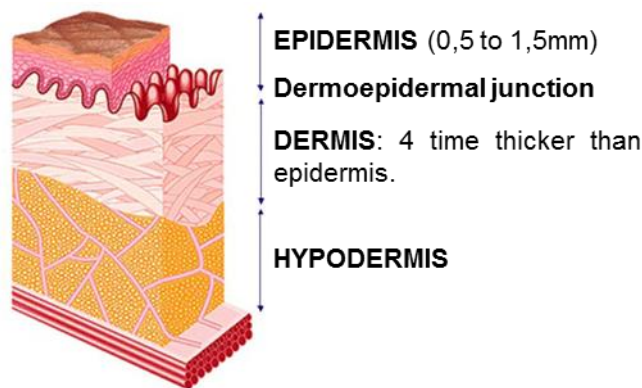
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# PART I: PRESENTATION OF THE RESEARCH AND DEVELOPMENT PROGRAM

## 1. SCIENTIFIC CONTEXT

The skin is the most extensive organ of the human body, since it represents 13% of the total mass of an adult and covers an average surface area of approximately 2 m<sup>2</sup>. A genuine interface with the external environment, the skin is far more than an envelope covering our bodies, it is the seat of many functions, which are as indispensable for life as they are complex. In the first instance, it ensures physical and biological protection of the organism against external aggressions, by forming an efficient barrier against the penetration of pathogenic agents and numerous xenobiotics. Thus, the penetration of active molecules contained in cosmetics is not controlled.

The anatomy of human skin is characterized by a complex multi-layer structure, comprising the epidermis, the dermis and the hypodermis, with each layer having different functions. The thickness of each of these varies according to its anatomical location, the individual's sex and age.



### 1.1. The skin's functions

#### 1.1.1. Protective function

The epidermis protects the skin from mechanical aggressions such as body movements and shocks.

The dermis is comprised of collagen and elastin fibers, which provide the skin with its tensile strength, the elastic properties thanks to which the skin returns to its original position after having been stretched.

The hypodermis protects the muscles and the underlying bones against shocks and pressure.

### 1.1.2. Environmental perception function

As a result of its wealth of sensitive fibers, the skin informs the organism about four main types of sensation: pain and temperature, touch and pressure.

### 1.1.3. Thermal regulation function

The skin helps to maintain our constant body temperature. Under cold conditions, the organism increase its metabolic rate. Under hot conditions, the active dilation of small dermal vessels promotes the evacuation of excess heat.

## 1.2. The skin's surface and its hydrolipidic film

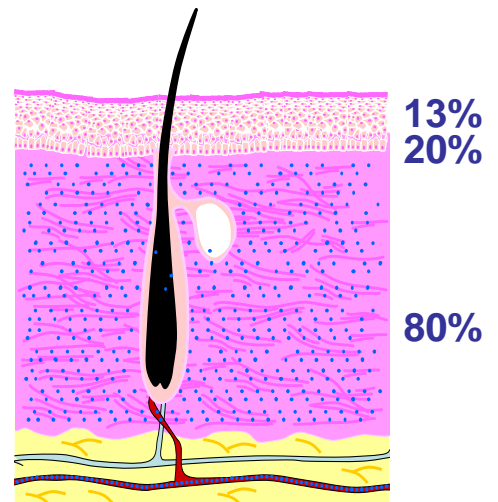
The hydrolipidic film is a protective film, which completely covers the surface of the epidermis. It comes from a mixture of sebaceous gland and sweat gland secretions, respectively corresponding to sebum and sweat. The sebum is a substance which tends to be oily and basic, whereas sweat is aqueous with an acidic pH. Its role is to:

### 1.2.1. Maintain moisture content

Every day, our organism loses 800 ml of water through the skin. This loss results mainly from the following two mechanisms:

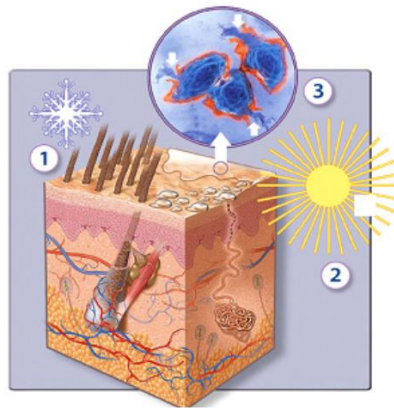
- Perspiration: the more or less abundant emission of water, following exposure to heat or resulting from a physical effort. This loss of water is related to the activity of the sweat glands and the vasodilatation of the skin pores.

- Insensible water loss: a permanent mode of body water elimination. Water is diffused from blood vessels towards the surface, passing through the dermis. This flow of water is referred to as the Insensible Water Losses (IWL). This evaporation process is naturally slowed by the presence of the hydrolipidic film at the skin's surface. The upkeep of this film and its qualities is thus essential to maintaining beautiful skin.



### 1.2.2. Protect the skin

The hydrolipidic film is also a protective film, isolating the organism from changes in temperature, pollution and bacteria.



## 2. THE STAKES OF THE R&D PROGRAM

The stakes of this research program are three-fold:

- Maintain beneficial molecules at the skin's surface, promoting the retention of water in the upper part of the epidermis, reinforce its cohesion and resistance, whilst at the same time curbing the penetration of exogenous substances (BIOVECTOR S).
- Promote the penetration of active molecules into the skin's middle layers (lower region of the epidermis and upper region of the dermis), and maintain them in this region (BIOVECTOR M).
- Promote the penetration of active molecules into the deep layers of the skin (lower region of the dermis, and hypodermis), and maintain them in this region (BIOVECTOR P).

### 2.1. GPS-S

#### 2.1.1. Reinforcing the skin's cohesion and resistance.

The sun, pollution, stress, lack of sleep,... every day the skin is subjected to difficult conditions by our sometimes frantic lifestyle.

Whether it be at work, at home or outside, the skin has no respite. Stress, fatigue, heating, air-conditioning, electronic devices, pollution, UV light, detergents, makeup, ... are all daily sources of cell damage. All of these represent various forms of oxidative stress, the primary cause of clinical ageing.

The skin is indeed the only organ (including the eye and part of the lungs), which is directly exposed to environmental toxins (pollution, tobacco, UV light...). All of these components, in particular the lipids in the hydrolipidic film, the proteins maintaining skin tone and elasticity, and the DNA of the nuclei and mitochondria, are subjected to toxic aggressions.

### 2.1.2. Maintaining active molecules at the skin's surface

Cosmetic products contain ever more effective and powerful active substances or ingredients. The molecules from which they are made diffuse extensively through the various layers of the skin, and can reach the bloodstream by systemic transmission, thereby limiting their efficacy and increasing their toxic risk.

### 2.1.3. Maintaining water at the skin's surface.

With age, the skin tends to become drier, since the sebaceous glands producing the hydrolipidic film (the sebum) at the skin's surface become less active.

There are various causes of a lack of skin hydration. Apart from age, skin dehydration depends on numerous other factors.

- A cold, dry, hot, or air-conditioned climate lowers the humidity of the air, and the drier the air, the more it absorbs evacuated humidity and dries the skin.

- Hot baths and the frequent use of soap affect the hydrolipidic layer.

- Exposure to wind and sun.

### 2.1.4. Sensitive and reactive skins

Since 1968, the proportion of individuals suffering from allergies of all types has risen to more than 30% of the population, and more than 60% of women consider their skin to be "sensitive". This sensitivity is the consequence of several frequently concomitant factors, such as an alteration of the barrier function, which causes a certain degree of skin permeability and an alteration of the skin's hydration. The skin is dry, loses its flexibility, its nociceptive sensitivity is increased, and the skin's tolerance threshold is lowered. This is when tingling, overheating, and inflammatory phenomena occur, with rashes, irritations and itchiness.

Not all dry skins are sensitive, however the lipidic deficit at the level of the inter-corneocyte is a decisive phenomenon in the incidence of reactive skins.

## 2.2. GPS-M and P

### 2.2.1. Promoting the transcutaneous displacement of active molecules.

The transcutaneous passage of a chemical compound, of any type, leads to successive steps of distribution and diffusion. A chemical compound is normally applied to the skin by means of a medium. This may be a simple solvent (water, a hydrophilic or lipophilic solvent), a gel, a viscous formulation, or a transdermal patch. The first skin permeation step is the

distribution between the medium and the hydrolipidic surface film. Indeed, only the molecules present at the interface between the two media can enter into the tissue. An active ingredient will thus diffuse less quickly in the adhesive of a patch than in a gel, resulting in delayed diffusion kinetics for the patch, associated with latency. Then, the molecule diffuses into the different layers of the epidermis, and continues to diffuse into the dermis. The diffusion from one layer to another is referred to as “permeation”. The diffusion can continue into the dermis and even into the subcutaneous tissue. Each step is characterized by its own speed, and the global speed of the process will, above all, depend on the slowest step.

The speed of transcutaneous absorption is a function of several processes, which can develop simultaneously:

- Diffusion of the active molecule in the medium
- Liberation of the molecule in its medium
- Change in the skin's degree of hydration
- Diffusion of the molecule into the corneal layer
- Diffusion of the molecule into the other skin layers.

#### 2.2.2. The different pathway through the stratum corneum

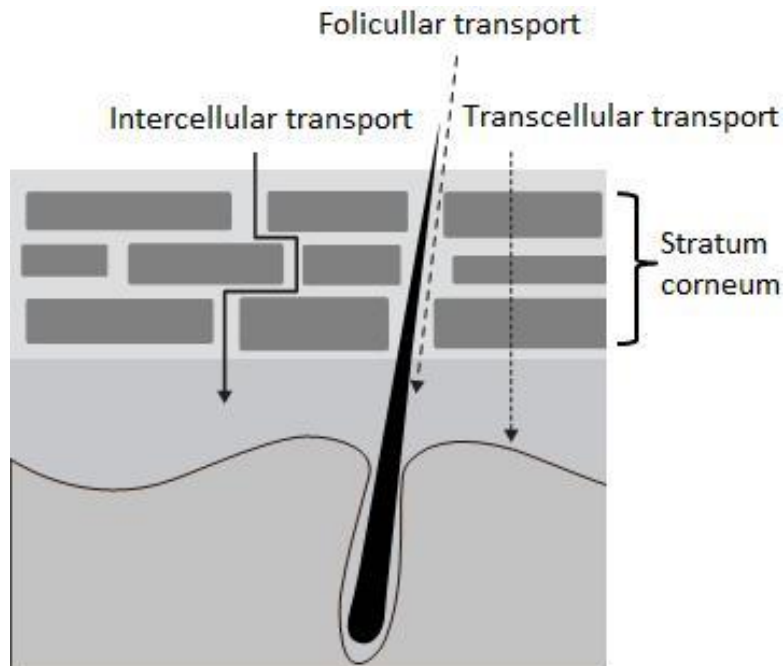
There are several pathways through the stratum corneum. Two main pathways can be identified: the intercellular pathway and the trans-cellular pathway are the two most commonly used pathway. A third, minor pathway is that making use of the skin appendages.

##### 2.2.2.1. *The intercellular pathway*

This is the pathway through the stratum corneum based on diffusion in the intercellular lipids (the lipids of the lipidic cement). Although the path used is longer and tortuous, this pathway is the fastest for many compounds, since they have a greater diffusion coefficient in the intercellular medium. This is a continuous lipidic medium, which is structured in double-layers. It thus offers both hydrophilic regions and lipophilic regions, which correspond to the pathway of lipophilic and hydrophilic compounds, respectively.

##### 2.2.2.2. *The transcellular pathway*

This pathway is used far more rarely. It is believed to make use of the path of the corneosomes, which produce bypasses between the cells and, since they are proteinaceous, can become sufficiently amphiphilic. In addition, the substances making use of this path must be able to integrate the double layer of phospholipids of which the cellular membranes are composed, in order to penetrate this layer. This path could thus be followed by amphiphilic or more or less lipophilic molecules.



#### 2.2.2.3. The adnexal path

As skin appendages represent approximately 1% of the skin's surface, they do not constitute one of the main paths. The adnexal path corresponds:

- to the transfollicular pathway. Since the pilosebaceous follicles are located in deep invaginations of the epidermis into the dermis, they can carry molecules to the reticular dermis. In addition, the pilosebaceous components do not have a highly developed stratum corneum, thus facilitating penetration at this location.
- to the eccrine gland pathway via the sweat duct. Although this possible, it has rarely been demonstrated. This is the case for aluminum chloride, which penetrates into the sweat ducts, and by sealing these decreases the flow of sweat. This compound is thus used as an anti-perspirant. However, the sweat gland pathway appears to be quite minimal, since the substances which can penetrate it must diffuse against the flow of sweat which rises to the skin's surface.

The active molecules present in cosmetic formulas can remain at the surface, or propagate homogeneously into the skin's different layers. The aim of this research program has been to develop biovectors (or "enhancers"), which are excipients added in small proportions to the formulation, which can allow the rate of absorption of the active ingredient to be increased or decreased.

### 3. THE CURRENT LIMITATIONS

Although it is possible to physically achieve a barrier effect, in particular with the help of hydrophobic products such as hydrocarbons (paraffin, Vaseline,...) or silicones, the discontinuous film they form at the skin's surface does not provide homogeneous protection and does not allow water to be retained in the upper layers of the epidermis. Hydrophobic molecules have no affinity for water produced by the sweat glands, and cannot spread homogeneously onto the skin's surface. As a consequence, if these molecules curb the imperceptible loss of water and limit the penetration of some molecules, they:

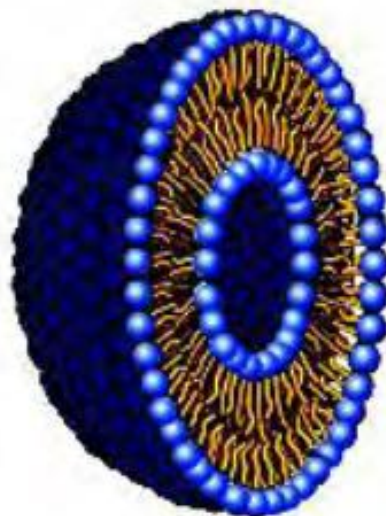
- Do not ensure cohesion of the keratinocytes
- Do not increase the resistance of the epidermis
- Do not allow water to be retained
- Do not retain the active molecules in the contact area

Conversely, state-of-the-art methods for conveying active ingredients are related exclusively to encapsulation systems.

Encapsulation includes all technologies allowing the preparation of individualized microparticles, consisting in a coating material containing an active ingredient.

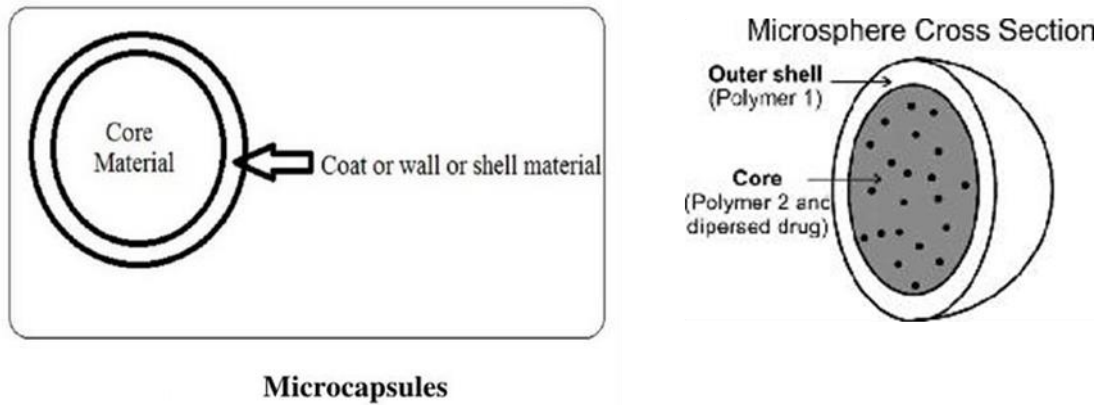
Encapsulation systems have a wide variety of internal structures. Depending on their structure, they can be classed into two main types:

- Vesicular systems: these are colloidal systems characterized by a hydrophilic or hydrophobic cavity, demarcated by a membrane made from an assembly of lipids, polymers or surfactant substances assembled in bilayers. Example of the liposome:



## - Particular systems

These consist in spherical particles consisting of a polymer matrix in which the active ingredient is dispersed or adsorbed.



These coating systems do not all have the same efficiency with all active ingredients. The technique thus needs to be adapted in accordance with the molecule to be encapsulated.

The formulation and industrial processes required to achieve this encapsulation are highly complex.

## 4. DESIGN OBJECTIVES

The design objectives are to develop three ingredients, having:

- A perfect affinity for the hydrolipidic film and the two affinity poles of the epidermis (lipid with the intercorneocyte cement, and aqueous with the keratinocytes), with the aim of:
  - Forming a continuous film at the surface
  - Reinforcing the skin barrier effect
  - Maintaining the substances in the contact zone
  - Limiting the passive diffusion of active molecules
  - Increasing the persistence effect
  - Curbing the insensible loss of water from the dermis to the upper layers of the epidermis
  - Producing a hydrating effect through hygroscopic action.
- The ability to disorganize the intercorneocyte lipids in such a way as to allow the active molecules to diffuse towards the middle layers of the skin. In cosmetic

applications, the guiding of active ingredients could allow the following goals to be achieved:

- An improvement in bioavailability
  - Increased penetrating power
  - Limitation of the molecules' passive diffusion
  - Decrease in the toxicity of the active ingredients
  - Reduction in the cost of products, through improvements in the dosage of the active ingredients
  - Maintain the substances in the contact zone
  - Increase the persistence effect
- The ability to disorganize the intercorneocyte lipids in such a way as to allow the active molecules to diffuse towards the deep layers of the skin. In cosmetic applications, the guiding of active ingredients could allow the following goals to be achieved:
  - An improvement in bioavailability
  - Increased penetrating power
  - Limitation of the molecules' passive diffusion
  - Decrease in the toxicity of the active ingredients
  - Reduction in the cost of products, through improvements in the dosage of the active ingredients
  - Maintain the substances in the contact zone
  - Increase the persistence effect

## PART II: RAW MATERIALS USED.

### 1. Cocamide MEA

#### 1.1. Description

INCI: Cocamide MEA

(CAS: 68140-00-1)

English names:

Amides, coco, N (hydroxyethyl)

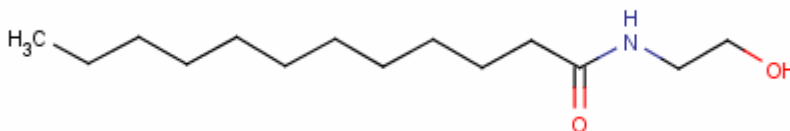
French names:

*Coconut de monoéthanolamine*

*Monoéthanolamine de coconut*

*Monoéthanolamine d'huile de noix de coco condensée*

This is obtained by condensing the fatty acids in coconut oil with monoethanolamine. Since coconut oil is made up from 50% lauric acid, the formula for cocamide can be written:  $\text{CH}_3(\text{CH}_2)_{10}\text{CONH}_2$ , even though the number of carbon atoms in the chain can vary.



Lauramide MEA, the main component of cocamide MEA, is produced by the condensation of lauric acid and monoethanolamine.

#### 1.2. Coconut oil

Coconut oil or copra oil is a plant oil manufactured from dried coconut kernel, referred to as copra.

Average composition in fatty acids:

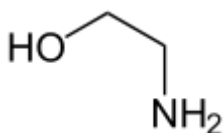
- Lauric acid	C12:0	44% - 51%
- Myristic acid	C14:0	13% - 18%
- Palmitic acid	C16:0	8% - 10%
- Capric acid	C10:0	6% - 10%

- Caprylic acid	C8:0	6% - 9%
- Oleic acid	C18:1	5.5% - 7.5%
- Stearic acid	C18:0	1% - 3%
- Linoleic acid	C18:2	< 2.5%

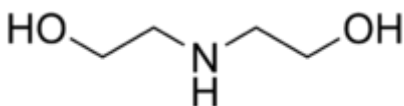
### 1.3. Diethanolamine and its derivatives

Diethanolamine, or DEA, is a chemical compound with the formula  $\text{HN}(\text{CH}_2\text{CH}_2\text{OH})_2$ . It is a colorless solid with an ammoniacal odor, which melts at 28 °C. It is a diol and a secondary amine, which is soluble in water and even hygroscopic.

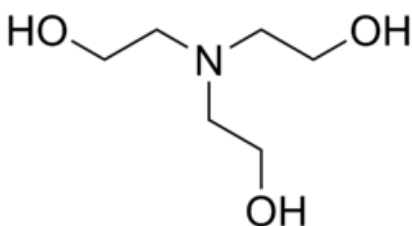
DEA is produced by reacting ammonia  $\text{NH}_4\text{OH}$  with excess ethylene oxide  $\text{C}_2\text{H}_4\text{O}$ , which successively leads to monoethanolamine (MEA), diethanolamine (DEA) and triethanolamine (TEA).



Structure of monoethanolamine



Structure of diethanolamine



Structure of triethanolamine

### 1.4. Plant oils and butters with a composition close to that of coconut oil

Coconut oil is rich in saturated fatty acids, lauric acid in particular.

#### 1.4.1. Plant oils and butters rich in lauric acid.

Lauric acid is rather uncommon in the plant kingdom, it can be found in bay laurel oil, in palm kernel oil, in tucuma butter and in murumuru butter.

#### 1.4.1.1. Bay laurel oil.

##### Composition of bay laurel oil:

- Essential polyunsaturated fatty acids: linoleic acid (23.80%), linolenic acid (0.83%).
- Monosaturated fatty acids: oleic acid (40.32%)
- Saturated fatty acids: **lauric acid (11.61%)**, palmitic acid (14.87%), stearic acid (1.55%), myristic acid (0.60%)

#### 1.4.1.2. Palm kernel oil

Palm kernel oil should not be confused with palm oil. These two oils are produced from the fruit of the palm tree *Elaeis guineensis*, however palm oil is produced from the fleshy part of the fruit (the mesocarp), whereas palm kernel oil is produced from the seeds. The compositions of these two oils are different.

##### Composition of palm kernel oil:

- Lauric saturated C12	<b>48.2%</b>
- Myristic saturated C14	16.2%
- Palmitic saturated C16	8.4%
- Capric saturated C10	3.4%
- Caprylic saturated C8	3.3%
- Stearic saturated C18	2.5%
- Oleic monounsaturated C18	15.3%
- Linoleic polyunsaturated C18	2.3%
- Other/Unknown	0.4%

#### 1.4.1.3. Murumuru butter

This butter is produced by pressing the seeds of the *Astrocaryum murumuru* tree, a palm tree originating from the Amazon.

##### Composition of Murumuru butter:

- Polyunsaturated essential fatty acids: linoleic acid	(omega 6)	(3.1%)
- Monounsaturated fatty acids: oleic acid	(omega 9)	(7.1%)

- Saturated fatty acids: **lauric acid (49.8%)**, myristic acid (23.5%), palmitic acid (5.8%), stearic acid (3.4%), capric acid (2.1%), caprylic acid (4.0%).

#### 1.4.1.4. *Tucuma butter.*

This butter, like Murumuru butter, is obtained from an Amazonian palm tree of the *Astrocaryum* genus, the *Astrocaryum tucuma*.

#### Composition of Tucuma butter:

- Polyunsaturated essential fatty acids: linoleic acid (omega 6) (3.15%)
- Monounsaturated fatty acids: oleic acid (omega 9) (10.60%)
- Saturated fatty acids: **lauric acid (46.28%)**, myristic acid (23.29%), stearic acid (5.54%), palmitic acid (5.99%).

#### 1.4.2. Plant oils and butters rich in saturated fatty acids.

##### 1.4.2.1. *Palm oil.*

Palm oil is produced from the pulp of fruit taken from the *Elaeis guineensis* palm tree.

#### Composition of palm oil:

- Essential, polyunsaturated fatty acids: linoleic acid (omega 6) (9.4%)
- Essential, monounsaturated fatty acids: oleic acid (38.4%)
- **Saturated fatty acids (48.8%):** palmitic acid (44.0%), stearic acid (4.4%)

##### 1.4.2.2. *Cocoa butter.*

Cocoa butter is produced by cold pressing the cocoa bean (*Theobroma cacao*).

#### Composition of cocoa butter

- Essential, polyunsaturated fatty acids: linoleic acid (omega 6) (3.3%)
- Monounsaturated fatty acids: oleic acid (33.2%)
- **Saturated fatty acids (59.2%):** stearic acid (33.0%), palmitic acid (26.2%)

##### 1.4.2.3. *Cupuaçu butter.*

Like cocoa butter, this butter is produced from the seeds of a tree from the *Theobroma* genus, the *Theobroma grandifolium*.

### Composition of Cupuaçu butter

- Essential, polyunsaturated fatty acids: linoleic acid (omega-6) (5.0%)
- Monounsaturated fatty acids: oleic acid (omega-9) (41.7%)
- **Saturated fatty acids (49%):** stearic acid (30.8%), arachidic acid (10.7%), palmitic acid (7.5%)

#### 1.4.2.4. *Sal butter or Borneo tallow.*

This is obtained from the kernel of the *Shorea robusta* fruit, a tree originating from South Asia.

### Composition of Sal butter

- Essential, polyunsaturated fatty acids: linoleic acid (omega-6) (2.1%)
- Monounsaturated fatty acids: oleic acid (omega-9) (37.30%)
- **Saturated fatty acids (55.6%):** stearic acid (42.7%), palmitic acid (12.9%)

#### 1.4.2.5. *Kokum butter.*

Also referred to as Kokum kernel fat, this butter is produced from the seeds of *Garcia indica*, an evergreen, which is found only in the rainforests of western India.

### Composition of Kokum butter

- Essential, polyunsaturated fatty acids: linoleic acid (omega-6) (4.08%)
- Monounsaturated fatty acids: oleic acid (omega-9) (34.96%)
- **Saturated fatty acids (51.83%):** palmitic acid (17.55%), stearic acid (34.28%),

#### 1.4.2.6. *Kpangnan butter.*

Also referred to as golden shea or Kanya butter, this is produced from the seeds of *Pentadesma butyracea*.

### Composition of Kpangnan butter

- Essential, polyunsaturated fatty acids: linoleic acid (omega-6) (4.40%)
- Monounsaturated fatty acids: oleic acid (42.30%)
- **Saturated fatty acids (47.7%):** stearic acid (40.00%), palmitic acid (7.70%),

#### 1.4.2.7. *Mango butter.*

This butter is produced by cold pressing the nuts from mango kernels.

### Composition of Mango butter

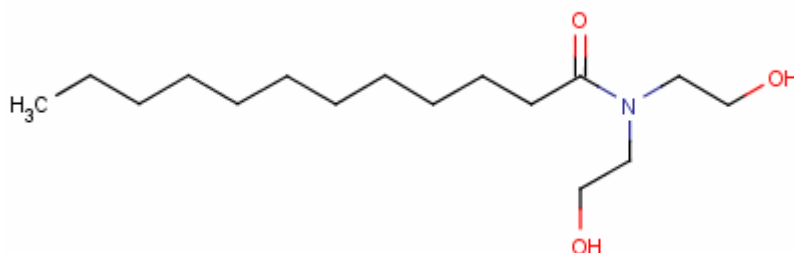
- Essential, polyunsaturated fatty acids: linoleic acid (omega-6) (4.56%)
- Monounsaturated fatty acids: oleic acid (omega-9) (39.87%)
- **Saturated fatty acids (54.56%):** iso-stearic acid (36.66%), palmitic acid (14.96%),

## 1.5. Cocamide derivatives used in cosmetics.

### 1.5.1. Cocamide DEA.

Cocamide MEA is obtained by condensing the fatty acids from coconut with diethanolamine (DEA).

Lauramide DEA, the main constituent of cocamide DEA, is produced by the condensation of lauric acid with diethylamine.



Cocamide DEA has the same properties as cocamide MEA.

### 1.5.2. Diethanolamides and monoethanolamides.

All condensations between a saturated fatty acid and a monoethylamine (MEA) or a diethylamine (DEA) lead to the production of a monoethanolamide or a diethanolamide, respectively. As coconut oil comprises different fatty acids (lauric acid, myristic acid, palmitic acid, capric acid, caprylic acid, and stearic acid), the condensation of each of these with DEA or MEA forms an ethanolamide, which may have the same properties as cocamide DEA.

**Example:** Lauramide DEA

*Function:* Antistatic / controlling agent of viscosity / synergy in foams / surfactants

*Role:* Reduces static electricity by neutralizing the electric charge present on a given surface./ Increases or reduces the viscosity of cosmetic products./ Improves the quality of foams produced by a system, by improving one or more of the following properties: volume,

texture and/or stability./ Reduces surface tension and promotes a uniform distribution of the product when it is used.

Lauramide DEA

*Function:*

Antistatic / controlling agent of viscosity / synergy in foams

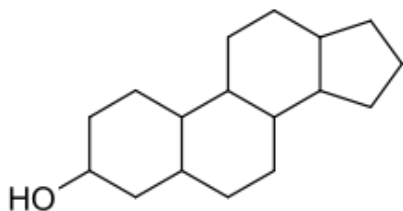
*Role:*

Reduces static electricity by neutralizing the electric charge present on a given surface. /Increases or reduces the viscosity of cosmetic products./ Reduces surface tension and promotes a uniform distribution of the product when it is used. /Improves the quality of foams produced by a system, by improving one or more of the following properties: volume, texture and/or stability.

## 2. Phytosterols

### 2.1. Sterols

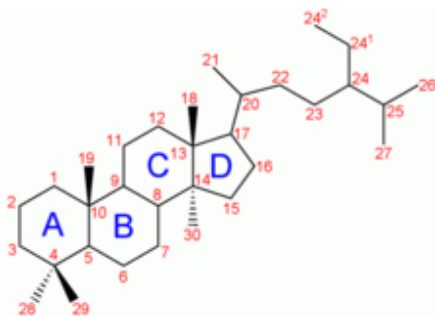
A sterol is a lipid having a sterane core of which the carbon 3 carries a hydroxyl group. Sterols are considered to be a subgroup of steroids.



Chemical structure of a sterol

### 2.2. Cholesterol

Cholesterol is a lipid from the sterol family, which plays a key role in numerous biochemical processes. The cholesterol molecule includes four carbon rings noted A, B, C and D (cyclopentano-perhydro-phenanthrene nucleus), and 8 asymmetric carbon atoms (carbon 3, 8, 9, 10, 13, 14, 17 and 20). Cholesterol has an -OH hydroxyl group on carbon 3 (C3), which is the hydrophilic component of cholesterol.

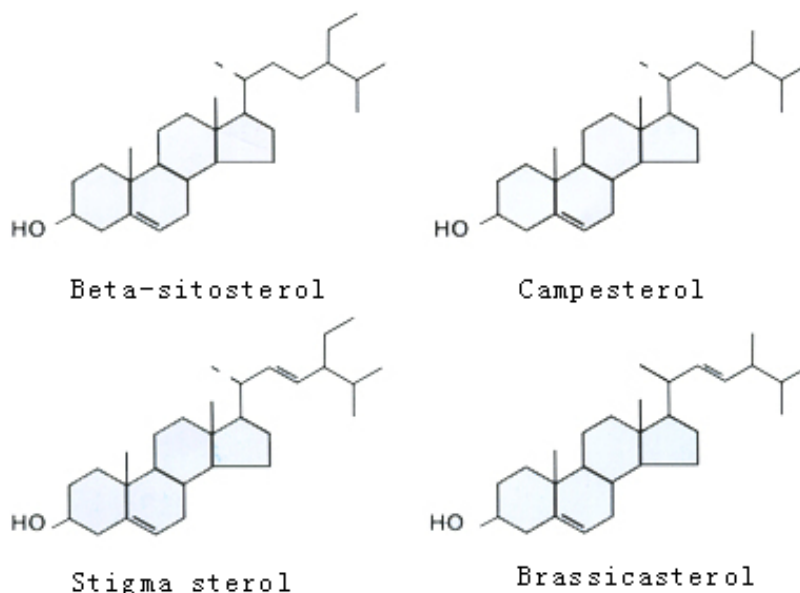


Structure and numbering of cholesterol.

Mammalian cells contain cholesterol, the role of which is to regulate the fluidity, permeability and metabolic properties of the membranes. Plant cells have a wide variety of sterols, in addition to cholesterol and phytosterols.

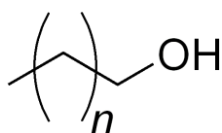
### 2.3. Phytosterols

Approximately 100 phytosterols are currently known. They belong to the triterpene family and differ from cholesterol by the presence of a double bond and/or a methyl or ethyl group at position C24 in the lateral alkyl chain. Some also carry an additional methyl group on the cyclic part of the steroid core. The most commonly encountered phytosterols, found in the cells of higher plants, are  $\Delta^5$ -phytosterols, characterized by the presence of a double bond between C5 and C6, at the level of the B-ring in the core. Among these, one can find  $\beta$ -sitosterol (45-95% of all sterols present in plants), stigmasterol, campesterol and brassicasterol.



### 3. Fatty alcohols

A fatty alcohol is a long carbonated chain having, in most cases, an even number of carbon atoms.



Chemical structure of linear fatty alcohols ( $n > 6$ ).

Fatty alcohols are widely used as detergents and surfactants since, thanks to their amphiphilic characteristic, they have surfactant properties.

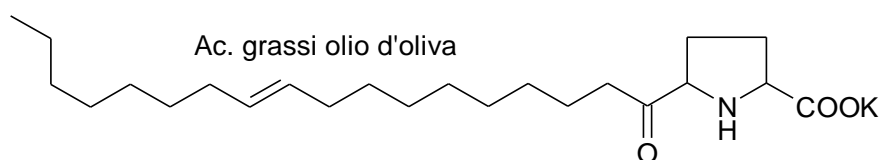
They are also used as emulsifiers, emollients and thickeners in cosmetics and the food industry.

## 4. Potassium olivoyl PCA

### 4.1. Description

INCI: POTASSIUM OLIVOYL PCA

Chemical structure



### 4.2. Manufacturing process.

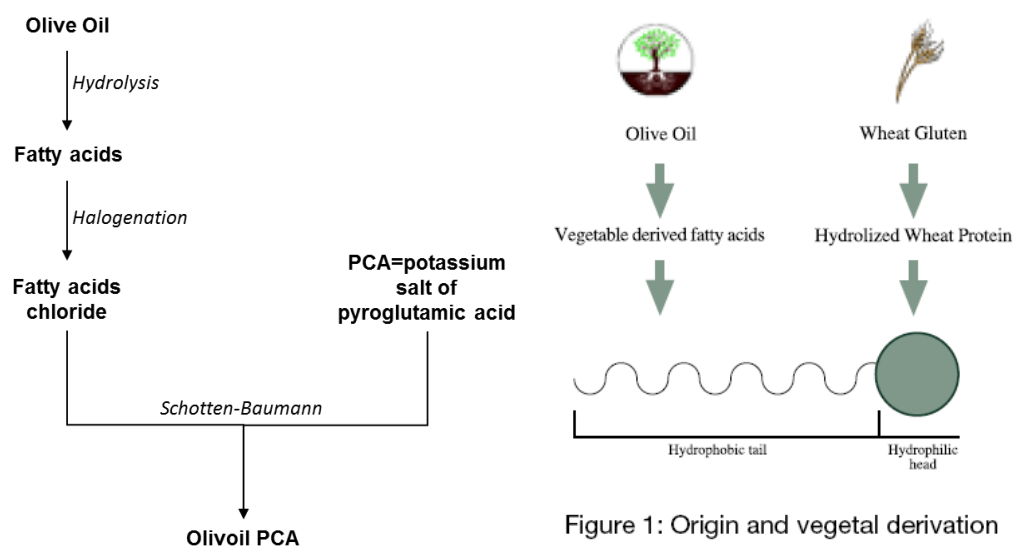


Figure 1: Origin and vegetal derivation

In this process, the PCA is obtained from wheat gluten, then from partially hydrolysed wheat protein.

### 4.3. Olive oil.

#### 4.3.1. Fatty acid composition.

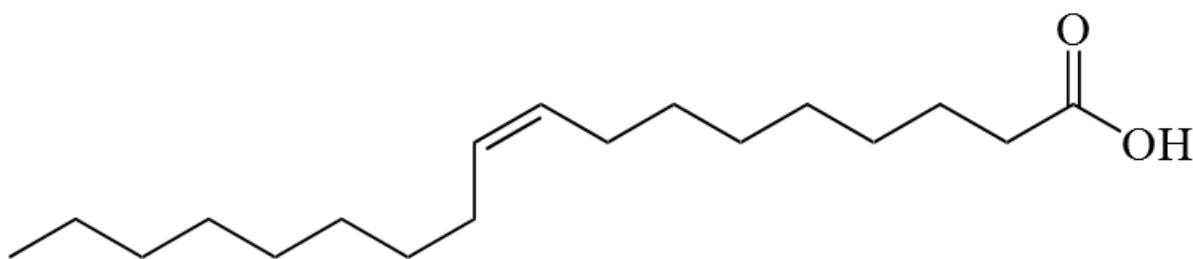
Fatty acids	Name	Mean	First quartile	Third quartile
C16:0	Palmitic acid	11.9	10.8	12.7
C16:1 $\omega$ 9	Hypogeic acid	0.12	0.11	0.14
C16:1 $\omega$ 7	Palmitoleic acid	0.8	0.61	1.07
C17:0	Margaric acid	0.08	0.05	0.12
C17:1 $\omega$ 9	Margaroleic acid	0.14	0.09	0.22
C18:0	Stearic acid	2.26	1.90	2.69
C18:1 $\omega$ 9	Oleic acid	72.2	68.9	75.0
C18:1 $\omega$ 7	Cis-vaccenic acid	2.28	1.95	2.69
C18:2 $\omega$ 6	Linoleic acid	8.35	6.64	10.7
C18:3 $\omega$ 3	Linolenic acid	0.65	0.59	0.70
C20:0	Arachidic acid	0.38	0.35	0.44
C20:1 $\omega$ 9	Gondoic acid	0.29	0.25	0.32
C22:0	Behenic acid	0.12	0.10	0.13
C24:0	Lignoceric acid	0.05	0.05	0.06
Saturated fatty acid		14.9	14.2	15.5
Monounsaturated fatty acid		76.1	72.9	78.6
Polyunsaturated fatty acid		9.02	7.28	11.5

#### 4.3.2. Oleic acid: the predominant fatty acid.

Oleic acid is named from the Latin *oleum*, which means oil. It is the most abundant long-chain monounsaturated fatty acid in our organism. Its basic chemical formula is C<sub>18</sub>H<sub>34</sub>O<sub>2</sub> (or CH<sub>3</sub>(CH<sub>2</sub>)<sub>7</sub>CH=CH(CH<sub>2</sub>)<sub>7</sub>COOH). Its IUPAC name is cis-9-octadecenoic acid, and its short lipid name is 18:1 cis-9. The saturated form of this acid is stearic acid.

#### 4.3.3. Structure of Oleic acid.

This is symbolized by the numbers 18:1 showing that it has 18 carbon atoms and an ethylene bond. It is preferred to indicate the position of the double bond, by using the number of carbon atoms between the last carbon atom (n° 18) and that of the double bond (n° 9), thus leading to 18-9, which is written n – 9, using n to designate the number of carbon atoms in the chain. Oleic acid is thus an unsaturated, more precisely a monounsaturated, fatty acid.



#### 4.4. PCA:

##### 4.4.1. Physico-chemical structure.

ID CARD	
	<b>Name:</b> pidolic acid
	<b>Synonym:</b> (S)-(-)-2-Pyrrolidone-5-carboxylic acid 5-oxoproline pyroglutamate PCA
	<b>Molecular weight:</b> 129,11 g.mol <sup>-1</sup>
	<b>Empirical formula:</b> C <sub>5</sub> H <sub>7</sub> NO <sub>3</sub>

From the chemical point of view, pyrrolidone carboxylic acid is the lactam of glutamic acid. It has an asymmetric carbon structure, hence the presence of two active forms (D and L) under polarized light, and a racemic form (DL).

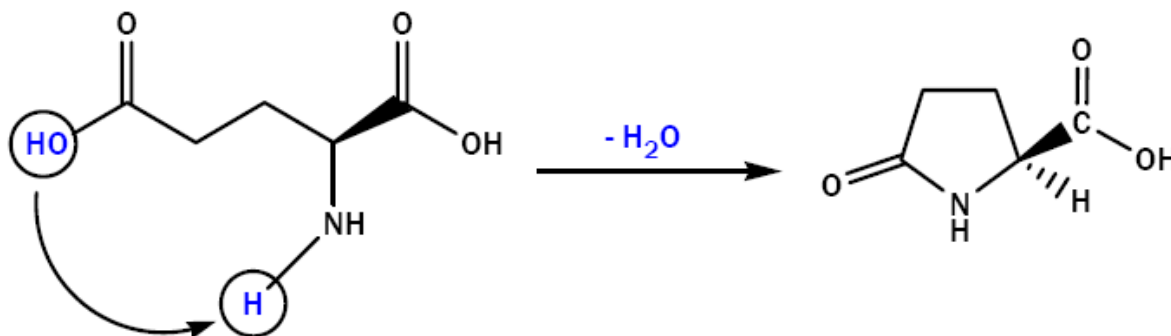
Pyrrolidone carboxylic acid (PCA) was discovered in 1883 by Haitinger, who observed that when it was heated, glutamic acid lost a water molecule, thus forming a new product, polyglutamic acid. The exact structure of the molecule was determined only in 1892, by Menozzi and Appiani.

In terms of beauty, Gertrud Pascher was the first, in 1956, to observe the high concentration of PCA in the human stratum corneum. Since that date, several studies have been made, dealing with the presence of this unusual metabolite in the human body. They revealed the presence of PCA in a free, acid or salt form, in most tissues and biological fluids of the body, thus confirming G. Pascher's hypothesis, according to which this molecule is found mainly in the stratum corneum (97% of PCA present in the skin is located in the stratum corneum), where it accumulates after differentiation of the epidermis.

PCA has a carboxyl function, which allows salts to be formed, from minerals in particular.

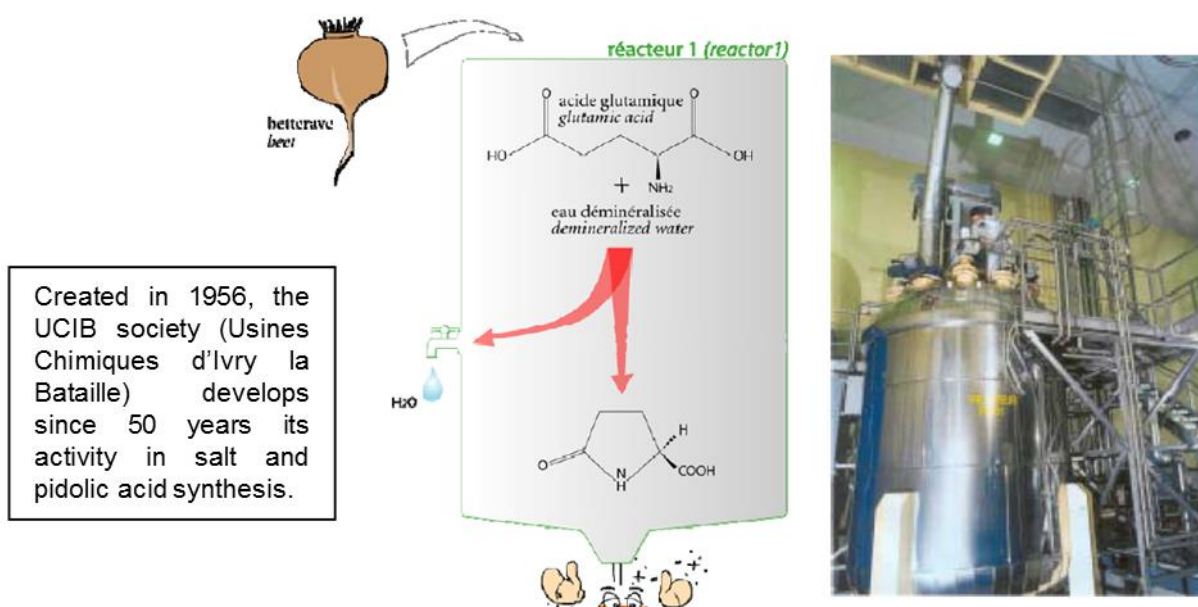
#### 4.4.2. Manufacturing process.

PCA is obtained from the thermal cyclization of glutamic acid (dehydration reaction). It is obtained exclusively in the L form, thanks to a chemical synthesis process allowing the molecule's stereochemistry to be perfectly controlled.



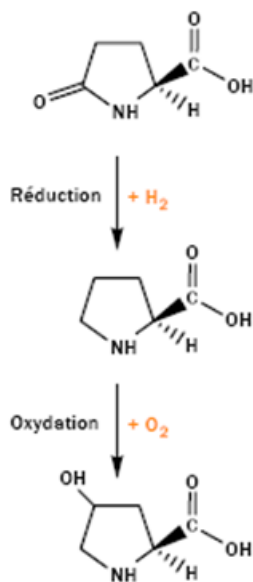
This process is very natural, because during the various steps, neither solvents nor chemical additives are used. For its part, glutamic acid is of plant origin, and is derived from sugar beet molasses.

The synthesis process is entirely automated. The various steps are automatically sequenced, which makes it possible to ensure a perfectly reproducible reaction and consistent product quality.



#### 4.4.3. Physiological roles.

PCA is a physiological molecule present in numerous tissues. Although 97% of PCA is found in the epidermis, the presence of L pyrroline carboxylic acid is observed in several organs such as the brain, the liver and biological fluids.



The L-PCA is a biochemical intermediate of compounds found in abundance in collagen: proline and hydroxyproline.

The proline (Pro) is a neutral non essential amino acid. It plays an important role in the proteins structure because it is involved in the curvature of the polypeptidic chain.

The hydroxyproline corresponds to the oxydated form of proline. It could be considered as a amino acid too, but unlike proline, it is not involved in proteins synthesis. It is found mainly in fibrous proteins of conjunctives tissues, in particular in elastine and collagen.

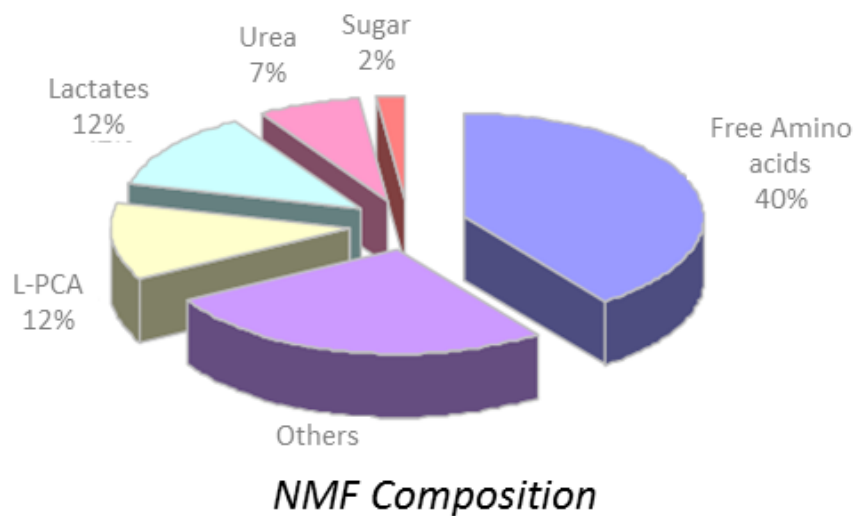
This information is particularly interesting in view of the fact that proline and hydroxyproline together represent approximately 21% of the constituent amino acids in collagen! The remainder is made up mainly from glycine (35%) and alanine (11%).

The abundance of proline/hydroxyproline is the reason for the rigidity and stability of collagen.

In the organism, and the skin in particular, pyroglutamic acid is an excellent differentiation agent of the epidermis, promoting the synthesis of epidermal lipids and the maturation of fillagrin.

#### 4.4.4. PCA and hydration.

The skin's moisturizing capacity is due to the presence, in the cells, of hygroscopic components, which are themselves hydro-soluble. This is a complex mixture of amino acids, organic acids, urea and mineral ions, referred to as the "Natural Moisturizing Factor" (NMF), which constitutes a real hydric reservoir within the skin. This is a very important ingredient, since correct hydration of the skin is the guarantee of a well-adjusted metabolism.



Among these components, L-PCA is the main catabolite of filaggrin (the name of which is derived from 'Filament Aggregation Protein'), a protein in the granular layer of the epidermis which, during the last phase of keratinization, is broken down into a mixture of amino acids.

Present in large quantities in filaggrin (27%), and then in NMF (12%), L-PCA is thus an excellent moisturizing constituent. Many studies have attributed this action to its hygroscopic power.

As skin hydration is vital to maintaining the elasticity and flexibility of the corneal layer, the action of L-PCA is of primary importance.

Since the epidermis does not have any enzymes responsible for its catabolism, L-PCA can disappear only through desquamation of the corneocytes, which explains its accumulation in the corneal layer (of which it represents 10% of the weight), and explains why there is ten times more L-PCA in the skin than in other tissues or organs, where the anabolism / catabolism balance is respected.

However, when a keratinization process is incomplete, the L-PCA level in the stratum corneum decreases. Healthy skin contains on average 180 mmol/g of L-PCA, a level below which the skin becomes dry and rough.

In addition to this unaesthetic and uncomfortable consequence, L-PCA depletion is also likely to weaken the barrier function exerted by the stratum corneum. When it is dehydrated, this structure can indeed crack and no longer act as an efficient barrier between our organism and the surrounding world.

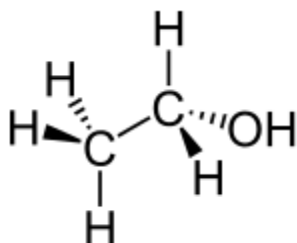
## 5. Ethanol

### 5.1. Description

INCI: ALCOHOL

CAS: 64-17-5

Chemical structure



### 5.2. Composition.

Denatured alcohol is generally ethyl alcohol, to which a denaturing agent is added, to make the mixture unfit for human consumption. Among the possible denaturing agents, one can cite methanol, diethylphthalate, thiophene, diethyl ether, isopropyl alcohol or even natural gas condensates.

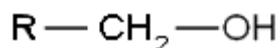
### 5.3. Substitution.

In organic chemistry, an alcohol is an organic compound in which one of the carbon atoms (this being tetrahedral) is associated with a hydroxyl group (-OH).

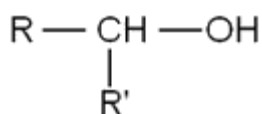
In generic terms, an alcohol thus contains the (R-OH) sequence, in which R is a variable organic radical, often an alkyl.

Depending on the nature of the carbon carrying the alcohol group, one can distinguish:

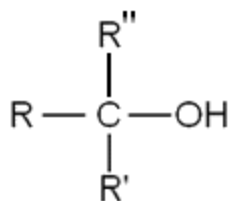
-primary alcohols, in which the carbon containing the hydroxyl group is associated with at least two hydrogen atoms and an organic radical R:



-secondary alcohols, in which the carbon containing the hydroxyl group is associated with one hydrogen atom and two organic radicals R and R':



-tertiary alcohols, in which the carbon containing the hydroxyl group is associated with three organic radicals R, R' and R'':



## PART III: PRODUCT DEVELOPMENT

### 1. GPS-S

#### 1.1. INCI composition.

N°CAS	N° EINECS/EUNCS	INCI Name	Chemical name IUPAC	Function	Quantity (International Code)
61789-40-0	263-058-8	COCAMIDOPROPYL BETAINE	1-Propanaminium, 3-amino-N-(carboxymethyl)-N,N-dimethyl-, N-coco acyl derivs., hydroxides, inner salts	Cleaning agent/Boosting foam	A
67762-27-0	267-008-6	CETEARYL ALCOHOL	Alcohols, C16-18 hexadecan-1-ol; octadecan-1-ol. SDA Reporting Number 19-060-00.	Emollient/Emulsifying agent/ Emulsion Stabilizer/Opacifying /Viscosity controlling	C
83-46-5	201-480-6	BETA-SITOSTEROL	Stigmast-5-en-3-.beta.-ol	Emulsion Stabilizer/Skin care/Stabilizer agent	C
68140-00-1	268-770-2	COCAMIDE MEA	Amides, coco, N-(hydroxyethyl)	Emulsifying agent/Emulsion Stabilizer/Surfactant/Viscosity controlling/Boosting foam	C
90045-98-0	289-964-3	SIMMONDSIA CHINENSIS (JOJOBA) SEED OIL	Simmondsia Chinensis Seed Oil is the fixed oil expressed or extracted from seeds of the desert shrub, Jojoba, Simmondsia chinensis, Buxaceae	Skin care/Emollient/ Hair conditioner/Abrasive	D
7732-18-5	231-791-2	AQUA	Water	Solvent	E

The quantitative analysis is provided in terms proportions, according to an international code: A = >50%; B = 25 to 50%; C = 10 to 25%; D = 5 to 10%; E = 1 to 5%; F = 0.1 to 1%; G = <0.1%; H = trace

#### 1.2. INCI GPS-S formula

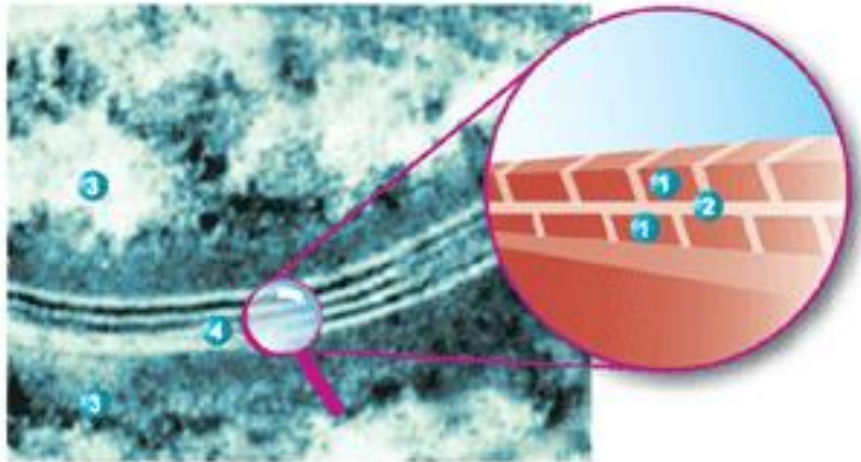
INCI: COCAMIDOPROPYL BETAINE - BETA-SITOSTEROL - CETEARYL ALCOHOL - COCAMIDE MEA - SIMMONDSIA CHINENSIS (JOJOBA) SEED OIL - AQUA

#### 1.3. Physico-chemical characteristics of BIOVECTOR S

pH	7 ± 1
Odor	Characteristic
Color	Off white
Viscosity	Cream
Microbiological standards	Total germs: <1000 UFC/g Molds: <100 UFC/g Yeasts: <100 UFC/g

#### 1.4. Synergy and recombination of ingredients

The epidermis is composed of different cellular layers. The outermost layer, the stratum corneum, is like a brick wall, providing a protective cover, resisting various agents of environmental aggression.



The stratum corneum is comprised of a deeply situated component called the Stratum compactum, whose cellular cohesion is ensured by the corneodesmosomes, as well as a more superficial layer, the Stratum disjunctum, which is lacking in desmosomes and is the seat of continuous desquamation. In this zone, the corneocytes are separated by intercellular spaces lined with a lipidic cement, which has a multiwall organization.

The physical barrier of the epidermis must be permanently maintained in good condition, in order to ensure that the skin is integrally protected.

The quality of the skin barrier is a function of the cohesion between the corneocytes and, therefore, between the different cellular layers, but also depends on the qualitative and quantitative composition of the intercellular cement.

#### 1.4.1. Molecular interactions under consideration

It is customary to classify skin lipids into neutral lipids (sterols; triglycerides, free fatty acids,...) and into phospholipid types of polar lipids (phosphatidylcholine, phosphatidylserine,...) and sphingolipids (Ceramides).

Their composition can vary according to the area of skin and the stage of keratinocyte differentiation.

At the time when they are expelled by the Odland bodies, the lipids tend to be polar and some of these are subjected to rearrangements during the differentiation process. The keratinocyte differentiation is furthermore correlated with lipid differentiation.

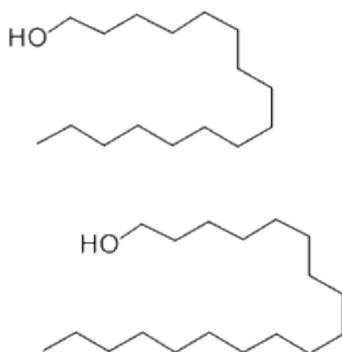
Transition from the Stratum granulosum to the Stratum corneum is thus accompanied by the almost total disappearance of the phospholipids and an increase in neutral lipids and ceramides.

Cutaneous lipids	Basal layer / Stratum spinosum	Stratum granulosum	Stratum corneum
Phospholipids	44.5	25.3	6.6
Cholesterol sulfate	2.6	5.5	2.0
Free sterol (cholesterol)	11.2	11.5	18.9
Free fatty acids	7.0	9.2	26.0
Triglycerides	12.4	24.7	Variable
Squalene	4.9	4.6	6.5
Sphingolipids	7.3	11.7	24.4
Glucosylceramides	3.5	5.8	Traces
Ceramides	3.8	8.8	24.4

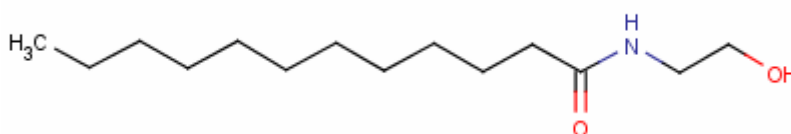
In any construction, more than the bricks, it is the cement, which ensures the stability and cohesion of the building. Bringing a cement to the skin, whose composition is close to that of the existing cement, would allow a “natural” barrier to be created.

Following extensive research, we chose to make use of an association of fatty alcohols, phytosterols and cocamide MEA.

Cetearyl alcohol was selected, since it is a fatty acid with a long carbon chain.



Monoethanolamine cocamide was selected, since it is comprised of a long carbon chain and an amide function.



The interaction between these two molecules: a fatty alcohol and monoethanolamine cocamide must allow a “ceramide-like” compound to be formed by bonding at the level of the amide function.

The barrier function of the epidermis is related to the presence and abundance of the Ceramides, in the multilayer structure of the intercorneocyte cement. In fact, the chemical composition of Ceramides influences the spatial presentation of the molecule and allows a better explanation to be given for their role in cellular cohesion. The more or less amphiphilic nature of Ceramides is thus the reason for their multilamellar organization. The Ceramides

have a primarily protective role, in addition to the biological and restructuring properties of the associated carbon chains.

#### 1.4.2. Scientific validation

In order to validate the formation of this “ceramide-like” molecule, we had a lipid specialist (ITERG) prepare the lipid dosage (sterols, ceramides and free fatty acids). As these substances (sterols, ceramides and free fatty acids) are absent from the initial composition, their presence in the final mixture should make it possible to validate the recombination produced by this association.

The following results were obtained:

Détermination des stérols	
Analyse réalisée le : 22/10/2012	
Stérols	Résultat(s)
Teneur en cholestérol	6,99 g/100g

Détermination de la teneur en céramides	
Analyse réalisée le : 05/10/2012	
Type	Résultat(s)
Teneur en céramides	3,7 g/100g

Détermination des acides gras libres	
Analyse réalisée le : 27/09/2012	
Type	Résultat(s)
8:0	9 mg/100g
10:0	25 mg/100g
12:0	567 mg/100g
14:0	198 mg/100g
15:0	2 mg/100g
16:0	105 mg/100g
18:0	46 mg/100g
18:1	80 mg/100g
18:2	15 mg/100g
Acides Gras libre totaux	1045 mg/100g

The significant quantity of ceramides found in the final mixture demonstrates the benefit to be obtained by associating these two components. This synergy provides tissue reconstruction and protection for the skin, thanks in particular to the addition of sterol, fatty acids and ceramides. The latter ingredients make up, for cholesterol 20% to 25%, for free fatty acids 15% to 20%, and for ceramides 50% of the corneal layer (Werts, 2006).

## 1.5. Formulation

**Solubility:** During formulation, BIOVECTOR S can be incorporated into the aqueous or the fatty phase, or even incorporated at the end of preparation, after production of the emulsion.

- When added to the aqueous phase, it provides the emulsion with a silky texture and highly slippery properties.
- When added to the fatty phase, it provides the emulsion with a drier, more powdery effect.
- When incorporated at the end of the preparation, it provides the cream with an additional nourishing appearance.

BIOVECTOR S can furthermore be incorporated into aqueous formulations (lotions, sprays, gels, ...) to which it will impart a toning effect.

BIOVECTOR S can be incorporated with silicones in order to provide it with nourishing properties.

When incorporated with anionic surfactants, it reduces their potential to irritate the skin.

**Dosage:** between 1% and 30%, or alone, depending on the type of product and desired efficiency.

## 2. GPS-M

### 2.1. INCI composition.

N°CAS	N° EINECS/ELINCS	INCI Name	Chemical name IUPAC	Function	Quantity (International Code)
ND	ND	PCA - OLEA EUROPAEA (OLIVE) FRUIT OIL	Condensate of olive oil fatty acids and potassium salt pyroglumatic acid	Moisturizer / Emoliant / Active	A
68140-00-1	268-770-2	COCAMIDE MEA	Amides, coco, N-(hydroxyethyl)	Emulsifying agent/Emulsion Stabilizer/Surfactant/Viscosity controlling/Boosting foam	C

Quantitative analysis given in terms of a proportion, according to an international code: A = > 50%; B = 25 to 50%; C = 10 to 25%; D = 5 to 10%; E = 1 to 5%; F = 0.1 to 1%; G = <0.1%; H = trace

### 2.2. INCI formula

INCI: PCA - OLEA EUROPAEA (OLIVE) FRUIT OIL - COCAMIDE MEA

### 2.3. Physico-chemical characteristics

pH	9 ± 1
Odor	Characteristic
Color	Pearl white
Viscosity	Cream
Microbiological standards	Total germs: <1000 UFC/g Molds <100 UFC/g Yeasts <100 UFC/g

## 2.4. Formulation

Solubility: During formulation, BIOVECTOR M can be incorporated into the aqueous or the fatty phase, or even incorporated at the end of production.

- When added to the aqueous phase, it promotes spreading of the cream onto the skin. It also provides the cream with a pearly appearance, without using pigments, improves its stability and increases its viscosity.
- When added to the fatty phase, the cream's nourishing effect is enhanced.

BIOVECTOR M can be incorporated into an emulsion and promotes the cream's penetration, whilst preserving its nourishing properties and surface smoothness.

BIOVECTOR M can be incorporated with silicones in order to provide it with thickening properties.

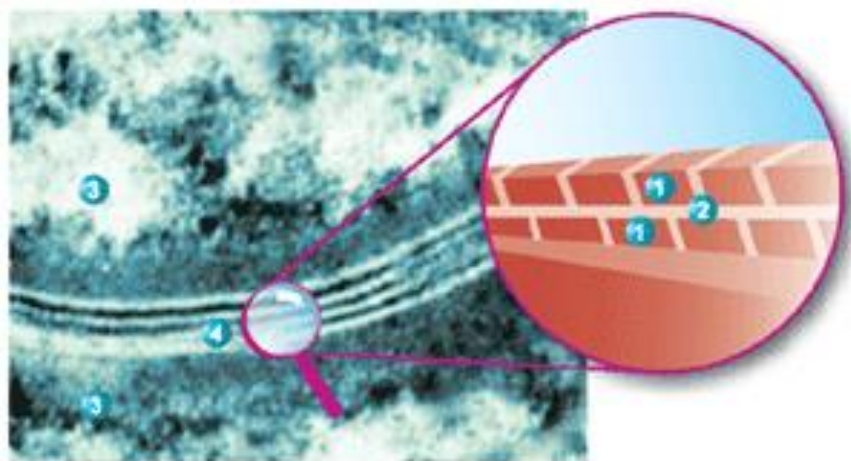
Dosage: between 1% and 10%, depending on the type of product and desired efficiency.

In addition to its technical and biovector properties, BIOVECTOR M stabilizes creams and thickens the mixture, providing it with a pearly appearance, and improves spreading onto the skin.

## 2.5. Synergy and recombination of the ingredients

The hydrolipidic film present at the surface of the stratum corneum is a water/oil emulsion. The water is represented by sweat secretions and the fatty acids are contributed by sebaceous secretions (96% of the lipids), sweat and keratinocyte degradation products produced by desquamation.

The stratum corneum is generally represented by the simple model of a wall of bricks.



In order to be able to penetrate into the lower layers of the skin, the active molecules must be able to squeeze their way between the cells, through the intercorneocyte lipids.

The median biovector promotes this intercellular passage. It involves passing through the stratum corneum by diffusion into the intercellular lipids (from the lipidic cement). Even though it is long and tortuous, this is the fastest path for many ingredients, since they have a higher diffusion coefficient in the intercellular medium. This is a continuous lipidic medium, which has a lipid bilayer structure. It thus provides both hydrophilic and lipophilic regions, corresponding to paths for lipophilic and hydrophilic ingredients, respectively. This is the main pathway for all active molecules.

The median biovector contains mainly fatty acids of the following types:

-Lauric acid: C12 :0

-Oleic acid: C18 :1

Fatty acids have been studied as molecules for the promotion of skin penetration, for the development of topical or transdermal applications containing different types of active substance. From the structural point of view, fatty acids consist of an aliphatic carbon and a terminal carboxyl group. They differ in the length of their chains, the number and position of their double bond(s) and their branching, if present. Their mechanism of action consists in reassembling distinct phases and forming pathway within the lipid bilayers. They are efficient at low concentrations and in numerous active ingredients. Furthermore, it is possible to modulate the promoting activity by means of modifications to the carbon chain. Researchers have studied the influence of the length of the fatty acid chain on the skin penetration efficiency of piroxicam. They have tested various fatty acids having a chain length ranging from C12 to C18. The skin promotion efficiency decreases with an increase in the length of the chain. The fatty acids having the shortest chains do not have sufficiently strong lipophilic properties to penetrate through the skin, whereas those with longer chains (longer than C18)

have such a strong affinity with the skin that they delay their own penetration into skin, as well as that of the active ingredients they carry.

It has been established that unsaturated fatty acids are better promoters of skin penetration than saturated fatty acids. Among the saturated fatty acids (capric, lauric and myristic acid), only lauric acid is efficient as a promoter of skin penetration. Linolenic acid, together with oleic acid, demonstrate the best efficiency in terms of the promotion of skin penetration, followed by palmitoleic, linoleic and arachidonic acids. Simple or double unsaturation also increases the transcutaneous passage. Fatty acids are promoters of skin penetration for lipophilic or hydrophilic ingredients.

The combination of fatty acids from the median biovector thus promotes the controlled penetration of active molecules. When associated with PCA, which increase the fatty acid's hydrophilic properties, these fatty acids also promote the penetration of active molecules. In fact, PCA is part of the NMF: Natural Moisturizing Factor. However, it is well documented that the amount of moisture in the skin has an influence on the permeability of the stratum corneum, and as a result this permeability can be modified simply by increasing the water content of the stratum corneum. Although the mechanism of action has not yet been determined, the combination of fatty acids with MEA plays a role in terms of permeability, since the level of penetration changes in the presence of this ingredient. It appears to play an important role in maintaining active molecules in the middle layers of the skin.

### 3. GPS-P

#### 3.1. Composition.

N°CAS	N° EINECS/ELINCS	INCI Name	Chemical name IUPAC	Function	Quantity (International Code)
ND	ND	PCA - OLEA EUROPAEA (OLIVE) FRUIT OIL	Condensate of olive oil fatty acids and potassium salt pyroglumatic acid	Moisturizer / Emoliant / Active	B
ND	ND	ALCOHOL	Denaturated alcohol	Solvent	B
7732-18-5	231-791-2	AQUA	Water	Solvent	D

Quantitative analysis given in terms of a proportion, according to an international code: A = > 50%; B = 25 to 50%; C = 10 to 25%; D = 5 to 10%; E = 1 to 5%; F = 0.1 to 1%; G = <0.1%; H = trace

#### 3.2. INCI Formula

INCI : ALCOHOL – PCA - OLEA EUROPAEA (OLIVE) FRUIT OIL - AQUA

### 3.3. Physico-chemical characteristics

pH	8.4 ± 1
Odor	Characteristic
Color	Colorless to light yellow
Viscosity	Liquid
Microbiological standards	Total germs: <1000 UFC/g Molds <100 UFC/g yeasts <100 UFC/g

### 3.4. Formulation

Solubility: During formulation, BIOVECTOR P can be incorporated into the aqueous or the fatty phase, or even incorporated at the end of the production.

- When added to the aqueous phase, it promotes penetration of the product, whilst preserving a dry touch.
- When added to the fatty phase, it promotes the product's penetration, whilst increasing its spreadability (recommended whenever waxes are used).
- When incorporated at the end of production, it significantly improves the cosmetic acceptability of even the thickest creams.

BIOVECTOR P can be incorporated with silicones.

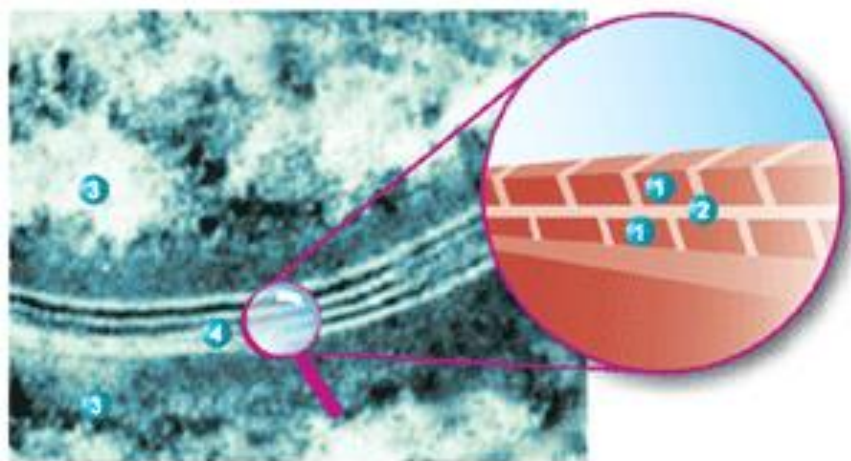
BIOVECTOR P can be added to surfactants in order to obtain a light and persistent foam.

Dosage: from 1% to 10%, depending on the type of product and desired efficiency.

### 3.5. Synergy and recombination of the ingredients

The hydrolipidic film present at the surface of the stratum corneum is a water/oil emulsion. The water is represented by sweat secretions and the fatty acids are contributed by sebaceous secretions (96% of the lipids), sweat and keratinocyte degradation products produced by desquamation

The stratum corneum is generally represented by the simple model of a wall of bricks.



In order to be able to penetrate into the deep layers of the skin, the active molecules must be able to squeeze their way between the cells, through the intercorneocyte lipids, and then penetrate the dermis.

The deep biovector promotes the intercellular and adnexal passage of the molecules. It involves passing through the stratum corneum by diffusion into the intercellular lipids (from the lipidic cement). Even though it is long and tortuous, the intercellular path is the fastest for many ingredients, since they have a higher diffusion coefficient in the intercellular medium. This is a continuous lipidic medium, which has a bilayer structure. It thus provides both hydrophilic and lipophilic regions, corresponding to paths for lipophilic and hydrophilic ingredients, respectively. This is the main pathway for all active molecules. Even though molecules do not frequently use them, the adnexal paths represent a direct pathway (they form a straight line along the hair shaft) towards the deep layers of the skin. The deep biovector also allows active molecules to use this path.

The deep biovector contains mainly fatty acids of the type:

-Oleic acid: C18 :1

Fatty acids have been studied as molecules for the promotion of skin penetration, for the development of topical or transdermal applications containing different types of active substance. From the structural point of view, fatty acids are composed of an aliphatic carbon and a terminal carboxyl group. They differ by the length of their chains, the number and position of their double bond(s) and their branching, if present. Their mechanism of action consists in reassembling distinct phases and forming pathway within the lipid bilayers. They are efficient at low concentrations and in numerous active ingredients. Furthermore, it is possible to modulate the promoting activity by means of modifications to the carbon chain. Researchers have studied the influence of the length of the fatty acid chain on the skin penetration efficiency of piroxicam. They have tested various fatty acids having a chain length ranging from C12 to C18. The skin promotion efficiency decreases with an increase in

the length of the chain. The fatty acids having the shortest chains do not have sufficiently strong lipophilic properties to penetrate through the skin, whereas those with longer chains (longer than C18) have such a strong affinity with the skin that they delay their own penetration into skin, as well as that of the active ingredients they carry.

It has been established that unsaturated fatty acids are better promoters of skin penetration than saturated fatty acids. Among the saturated fatty acids (capric, lauric and myristic acid), only lauric acid is efficient as a promoter of skin penetration. Linolenic acid, together with oleic acid, demonstrate the best efficiency in terms of the promotion of skin penetration, followed by palmitoleic, linoleic and arachidonic acids. Simple or double unsaturation also increases the transcutaneous passage. Fatty acids are promoters of skin penetration for lipophilic or hydrophilic ingredients.

The use of type C18 :1 fatty acids in the deep biovector thus promotes the controlled penetration of active molecules. When associated with PCA, which increase the fatty acid's hydrophilic properties, these fatty acids also promote the penetration of active molecules. In fact, PCA is part of the NMF: Natural Moisturizing Factor. However, it is well documented that the amount of moisture in the skin has an influence on the permeability of the stratum corneum, and as a result this permeability can be modified simply by increasing the water content of the stratum corneum.

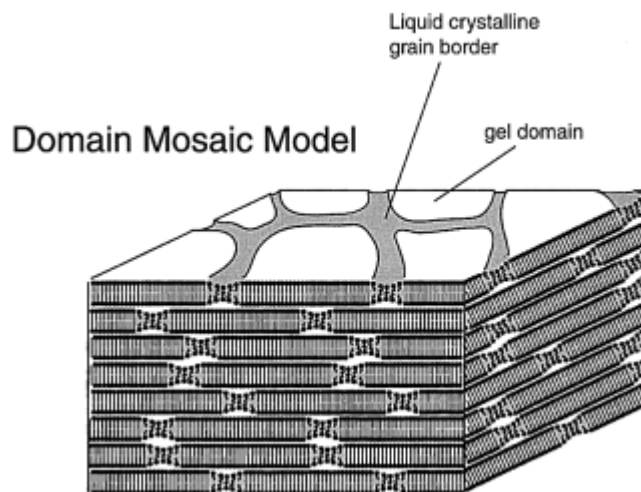
In most cases, the solvent has a strong influence on the efficiency of fatty acids; when they are associated with fatty acids, solvents such as propylene glycol or ethanol promote synergy of the skin penetration phenomenon. Ethanol, associated with the fatty acids of olive oil, combined with PCA, allows the molecules to be directed down to the deep layers of the skin.

## PART IV: EXPERIMENTAL VALIDATIONS

### 1. GPS-S

#### 1.1. SKIN PROTECTION.

Despite its minimal thickness of approximately 10  $\mu\text{m}$ , the stratum corneum is the skin's main protective element, thanks to its specific structure. The corneocytes, keratinized and highly elongated cells, overlap each other like the tiles on a roof, and are almost totally dehydrated. Keratin is a hydrophilic protein which provides them with a very high degree of resistance. These cells are joined together by a cement made from a mixture of polyunsaturated fatty acids, cholesterol and ceramides. In addition, their cohesion is ensured by corneosome protein bonds. It is this compact, dense structure which slows, or even prevents, the diffusion of exogenous substances, and slows the diffusion of water originating in the dermis. It should be noted that this function relies on the cohesion of the stratum corneum.



##### 1.1.1. Measurements

In order to measure the usefulness of BIOVECTOR S in the field of skin protection, we exposed, *in vivo*, the surface skin cover to an exogenous substance in the form of a dye, in order to measure, with and without BIOVECTOR S, the degree of penetration, thus making it possible to express any possible differential effect. In this context, the following experiment was carried out.

##### 1.1.2. Protocol

- cleaning of the skin with a detergent (3 drops of PAIC dish-washing liquid)
- application of 95° alcohol (5 ml using a spray)

-**T0**: application of 150 mg of the pure tested ingredient (Biovector S oil).

-wait for 1 minute

-**T1**: deposit two drops of aqueous dye on the treated zone (methylene blue diluted in water to 10 g/l).

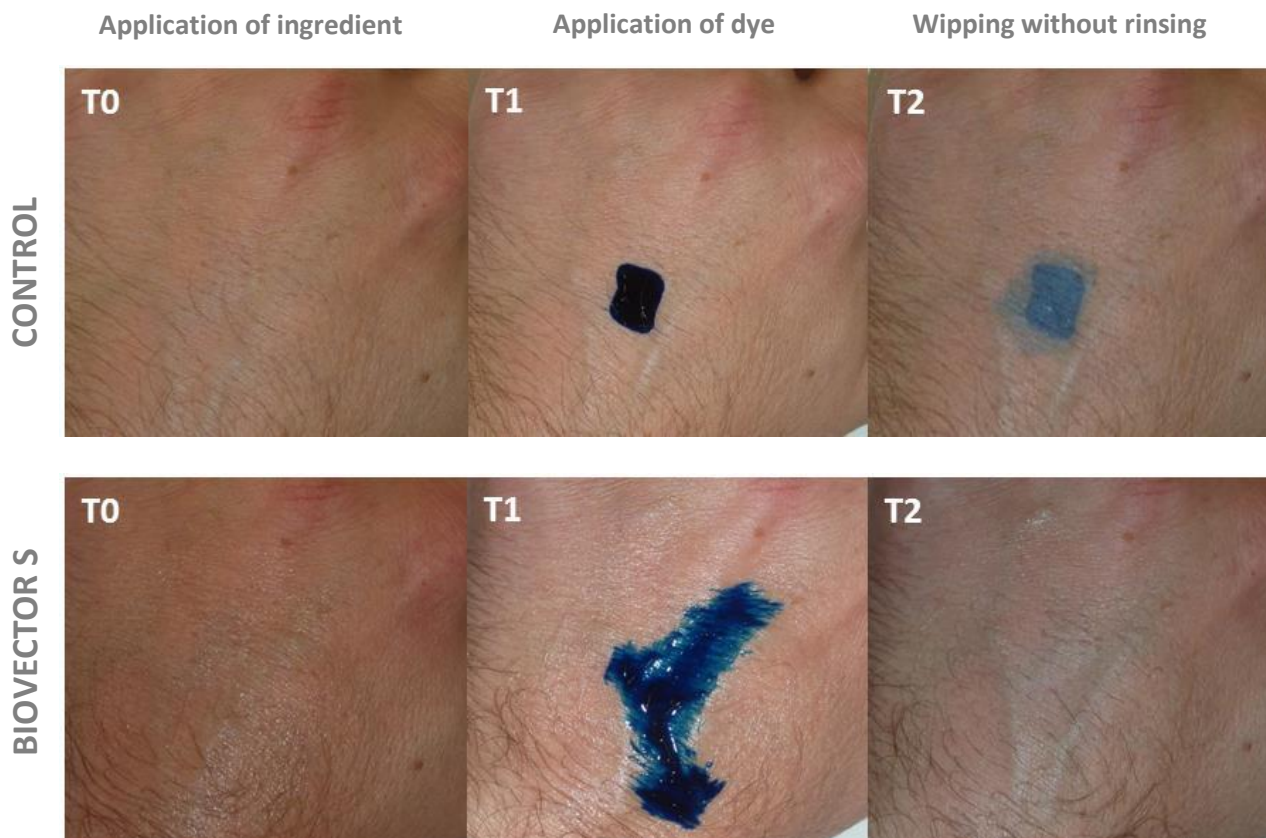
-wait for 1 minute

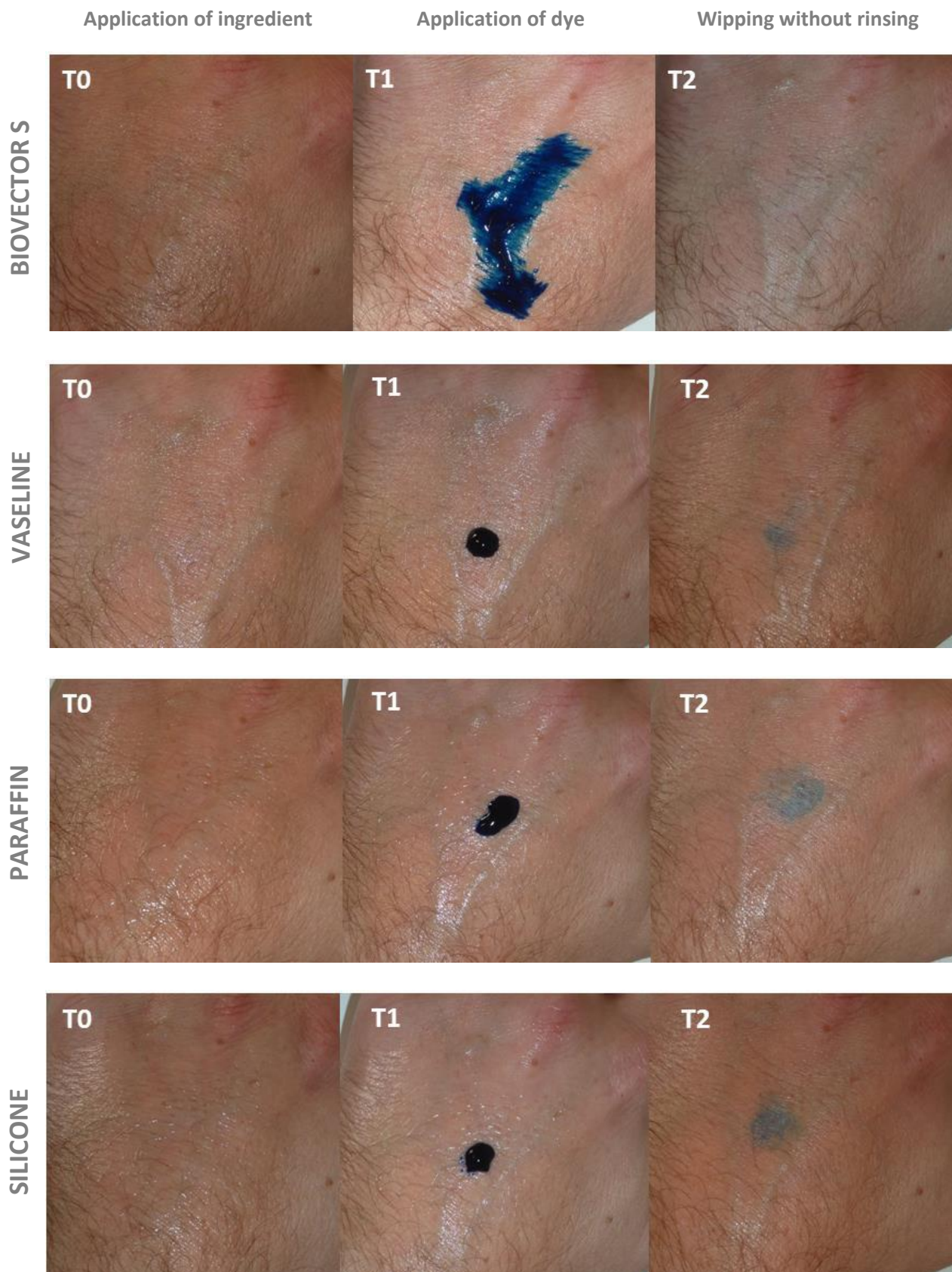
-**T2**: drying with absorbent paper, without rinsing.

-photographic recording

-comparison with reference hydrophobic products (vaseline, paraffin, silicone) under the same experimental conditions.

### 1.1.3. Results





#### 1.1.4. Interpretation

##### 1.1.4.1. *A continuous and cohesive film*

The differential existing between the control, the reference protective products, and the studied product, in particular at the level of the interfacial tension at T1 (shape of the drop), provide insight into the affinity of BIOVECTOR S for the skin epidermis, whether it be considered with respect to the hydrophilic protein cells, or the intercorneocyte lipids. The film deposited on the skin is a continuous and cohesive film with a high cutaneous affinity.

In the presence of BIOVECTOR S, the drop deposited on the skin encounters no form of resistance, contrary to the comparative case. The micro-depressionary relief and the opposite affinity of the intercorneocyte and keratinocyte lipids produce, in the control and the comparative cases, a high degree of surface tension.

The contact angle between the skin surface and water is a parameter indicative of the hydrophilic or hydrophobic tendency. The skin tends to be a hydrophobic surface. Curiously, the drier the skin, the more hydrophobic it becomes. (AFIFI Y, ELKHYAT A, HASSAM B, HUMBERT Ph).



##### 1.1.4.2. *Optimal protection*

Although they provide interfaces, which are efficient and have a low absorbability, reference hydrophobic products do not provide sufficient protection with respect to exogenous factors. As they have no affinity for water and some have a weak affinity for oil, they can be deposited on the skin's surface in the form of more or less significantly sized droplets, between which exogenous substances are able to penetrate. This phenomenon is confirmed by the experiment described in the following:

Two drops of dyed water have been added to a vial containing the ingredient diluted in oil (10% ingredient and 90% oil). The mixture has been shaken, leading to the following results.



**BIOVECTOR S**

**VASELINE**

**PARAFFIN**

**SILICONE**

## 1.2. MOISTURIZING EFFECT.

The dermis is the skin's main water reservoir. From the basal layer to the granular layer, the human epidermis contains 65% to 70% water. However, this proportion decreases at the level of the stratum compactum, where it is only 40%. Meanwhile, the superficial layer of the corneal layer contains only 15% of water, and this decreases to 7% in elderly persons.

Several processes can be implemented in order to maintain a well moisturized skin:

### 1.2.1. Reducing evaporation.

Evaporation is restricted by intercellular cement. Water, polar molecules and lipophilic substances can cross the stratum corneum only by passing through the intercellular spaces. As the corneocytes have a membrane which is impermeable to water, the latter must use a tortuous path, over a much greater distance than that corresponding to the thickness of the corneum stratum itself. As a consequence of their chemical structure, it is the ceramides which play a key role in regulating the flow of transepidermal water. These amphiphilic molecules form sheets, arranged in lipidic bilayers, which create a highly stable lamellar structure, reinforced by the presence of long carbon chains and hydrogen bonds connecting the ceramides together.

By referring to the composition of intercorneocyte lipids, one notices in particular that it is very low in phospholipids, and is composed mainly of ceramides (50%), cholesterol (20%-25%) and free fatty acids (15%-20%) (Wertz, 2006). It is this intercorneocyte cement which plays an essential role in the hydric barrier, by reducing losses in the organism's water content.

However, the measurements carried out on BIOVECTOR S by ITERG reveal a similarity in composition, in particular:

<b>Détermination des stérols</b>	
Analyse réalisée le : 22/10/2012	
Stérols	Résultat(s)
Teneur en cholestérol	6,99 g/100g

<b>Détermination de la teneur en céramides</b>	
Analyse réalisée le : 05/10/2012	
Type	Résultat(s)
Teneur en céramides	3,7 g/100g

<b>Détermination des acides gras libres</b>	
Analyse réalisée le : 27/09/2012	
Type	Résultat(s)
8:0	9 mg/100g
10:0	25 mg/100g
12:0	567 mg/100g
14:0	198 mg/100g
15:0	2 mg/100g
16:0	105 mg/100g
18:0	46 mg/100g
18:1	80 mg/100g
18:2	15 mg/100g
Acides Gras libre totaux	1045 mg/100g

Under these conditions it can easily be understood that, by reinforcing the intercorneocyte lipid content, BIOVECTOR S reduces evaporation, through a two-pronged effect:

- by reinforcing the hydrolipidic film at the surface of the epidermis
- by reinforcing the intercorneocyte lipids between the hydrophilic protein cells

These phenomena are inexistent with reference hydrophobic products, which spread discontinuously over the skin's surface, without being integrated into hydrophilic film, nor into the intercorneocyte lipids.

#### 1.2.2. Increasing the fixation of water during its passage through the stratum corneum.

Apart from efficiently preventing the insensible water loss (IWL), through a two-pronged effect (hydrolipidic film and intercorneocyte lipids), the ability of BIOVECTOR S to bind water molecules is a considerable asset, for many reasons:

- Significant increase in its moisturizing properties
- Impact on the skin's softness and suppleness
- Maintaining active molecules in the contact zone
- Reducing passive diffusion
- Reducing the risks of toxicity

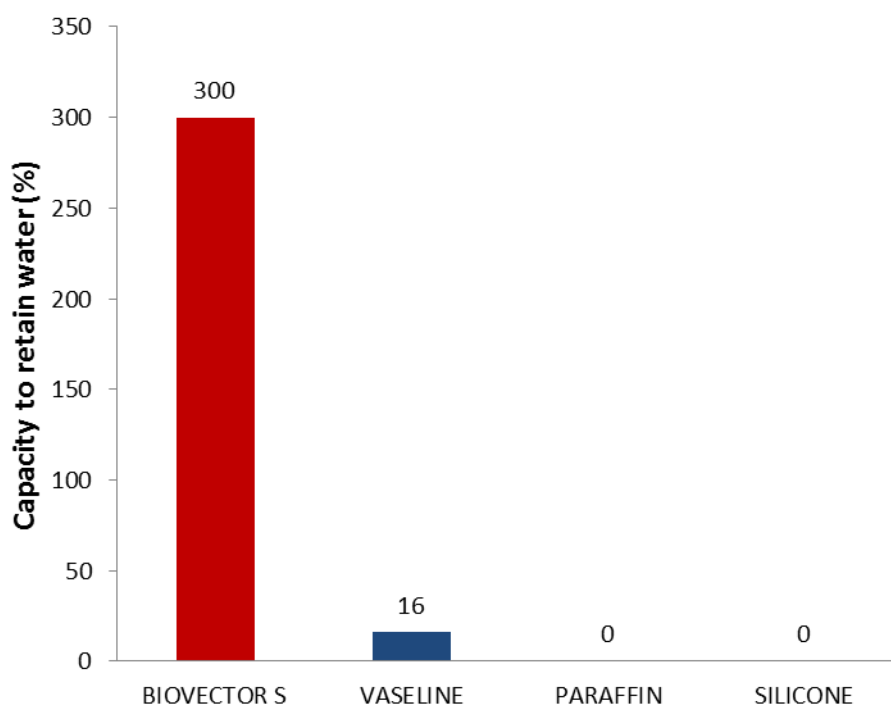
##### 1.2.2.1. *Measurements*

In order to measure the advantage of BIOVECTOR S in terms of skin hydration, we added increasing quantities of water to the different tested ingredients. This allowed us to measure the hygroscopic power of BIOVECTOR S, when compared to other reference products. In this context, the following experiment was carried out.

##### 1.2.2.2. *Protocol*

- In a 50 ml beaker, 10 ml of the tested ingredient is added (BIOVECTOR S, Vaseline, paraffin, silicone)
- Addition of water, drop-by-drop, and stirring with a spatula
- When the ingredient and the water are no longer miscible, small droplets remain.
- For each ingredient, the volume of water needed to attain non-miscibility is noted.
- This volume of water is compared with the volume of ingredient (10 ml), in order to determine its ability to retain water (percentage).

### 1.2.2.3. Results



### 1.2.2.4. Interpretations

BIOVECTOR S is able to retain 5 times its own weight of water, which is far more than can be achieved with reference hydrophobic products. It is this proven quantity, which makes BIOVECTOR S a reference product.

### 1.2.3. Promotion of cohesion between keratinocytes and reinforcement of the skin's resistance to physical or mechanical alteration

Various experiments have shown that when an adhesive is used to successively remove layers of the stratum corneum (referred to as "stripping"), the skin's total permeability increases. The same applies in the case of burns, abrasion, or scarification. In the case of an excessively dry skin, a loss in cohesion between corneocytes leads to an increase in the loss of water (i.e. the IWL increases).

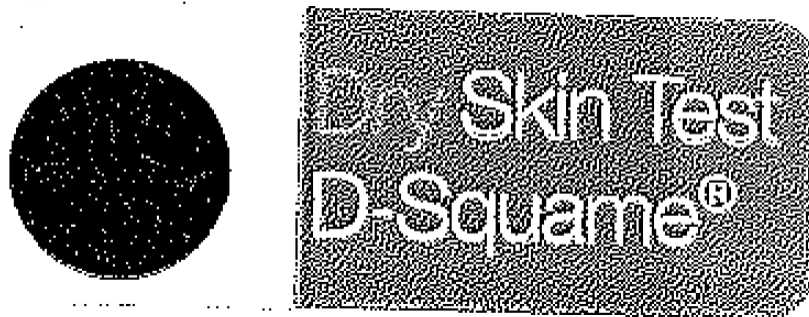
#### 1.2.3.1. Measurements

The usefulness of a protective product depends partly on its ability to reinforce the cohesion between keratinocytes and to resist physical or mechanical alterations, with the aim of limiting the tissue's permeability to exogenous substances.

In order to evaluate the corneocyte cohesion, we used Dsquames (Monaderm).



D'Squame® is an adhesive patch which is attached to the skin and held in place for 30 seconds. It removes the first superficial layers of the skin. The Dsquake scan (RICOH MP C 3002 – 200 dpi resolution) allows an image to be taken, in which the keratinocytes are represented by white spots.



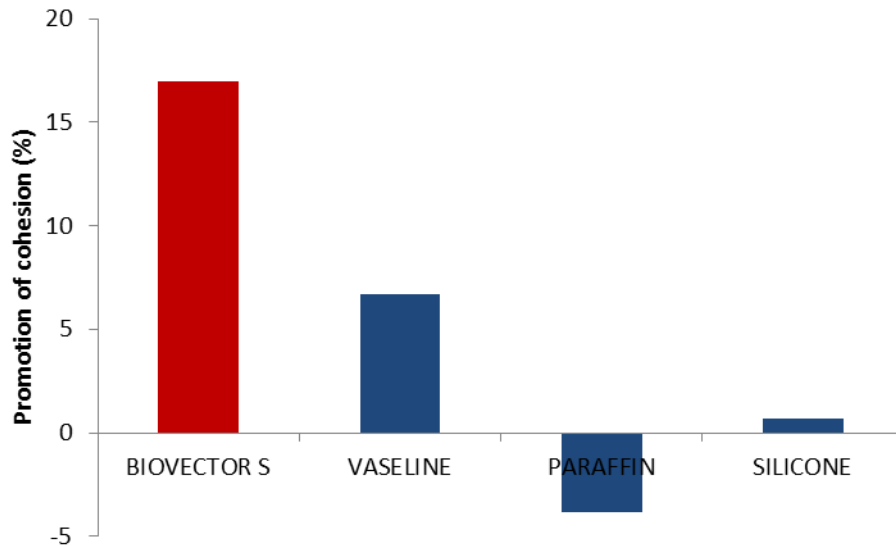
#### 1.2.3.2. Protocol

- For each volunteer, the pure tested ingredients were applied to the dorsal side of the left forearm (zone close to the elbow, where the skin is particularly dry). The matching zone on the right arm was used as a control.
- After 15 minutes, the zones were rinsed with a detergent (3 drops of PAIC dishwashing liquid).
- Fifteen minutes after rinsing, a Dsquake patch was applied to the skin for 30 seconds, on the zone where each ingredient had been deposited (left arm). The corresponding control tests (right arm) were also carried out.
- The Dsquake patches were then scanned and the white spots were counted.

#### 1.2.3.3. Results

The following graph indicates the loss (positive %), or gain (negative %) in percentage of keratinocytes found on the Dsquake patch after application of the ingredient. Thus, with BIOVECTOR S, 17% less cells were found on the Dsquake patch than with the control.

### PROMOTION OF COHESION BETWEEN KERATINOCYTES



#### 1.2.3.4. Interpretations

Although reference hydrophobic products remain at the skin's surface, if they have a low affinity with its tissues and consequently represent a difficult to absorb physical interface, they are moderately useful with respect to physical or mechanical alterations.

Tissues which have been pretreated with BIOVECTOR S have a greater resistance to stripping, and the cohesion of the keratinocytes is greater than that of reference hydrophobic products:

- +12% with respect to vaseline
- +25% with respect to paraffin
- +19% with respect to silicone

#### 1.2.4. Cumulative moisturizing effect

With respect to its composition, its dual affinity, the film it forms at the skin's surface, its ability to retain water, and its cohesive power with respect to the keratinocytes, its global moisturizing power can easily be compared with reference hygroscopic moisturizing molecules, such as sorbitol and glycerine together, or hyaluronic acid.

##### 1.2.4.1. Measurements

Corneometry is used to evaluate the hydration of the superficial layers of the skin. This method is based on the relationship existing between the tissues' electrical properties and their water content.

In order to evaluate the influence of various products on skin hydration, we implemented a well-defined procedure. The measurement points were identified by marks (spots) made on both arms with a red felt pen (a total of 30 points). As skin hydration can vary strongly as a function of the measurement zone, it was essential to carry out the before-and-after measurements at exactly the same position. At each of these 30 points, the skin hydration was measured using the MDS 800 Multi Dermoscope®.



The MDS 800 Multi Dermoscope is multifunctional and can measure: the water content of the upper layer of skin, the cutaneous elasticity, the cutaneous pigmentation and the type of skin. This device is widely used in dermatology, for the evaluation of various parameters.

This device is equipped with sensors allowing the degree of skin hydration to be measured. It uses a half-moon array of diodes to indicate the skin's hydration as a percentage.

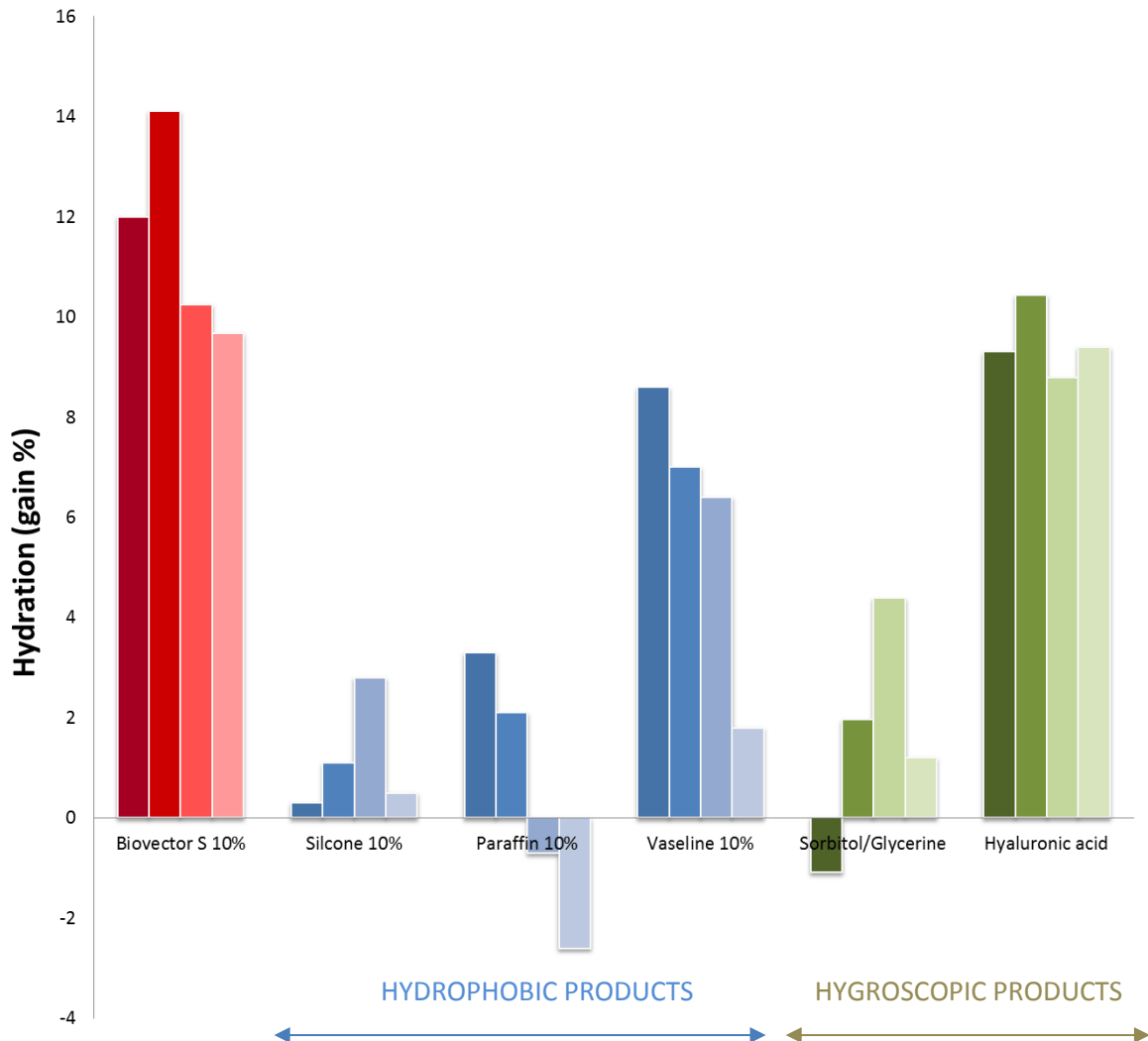
#### 1.2.4.2. Protocol

For each of these 30 points, we measured the level of hydration, before applying any cream. The creams were then applied. The levels of hydration were then measured, 1, 2, 5 and 7 hours after application of the cream(s).

In order to avoid any variability arising between different areas of skin (arm, forearm, and hand), we chose to express the results by determining the difference between the measurements made before and after application of the cream.

#### 1.2.4.3. Results

The results are shown below, with BIOVECTOR S in red, the hydrophobic products in blue, and the hygroscopic products in green. For each of the ingredients, the level of hydration is shown (from the darkest to the lightest shade of each color), 1, 2, 5 and 7 hours after application.



#### 1.2.4.4. Interpretations

Hygroscopic products such as sorbitol, glycerine or hyaluronic acid have a hydrating power which is greater than that of hydrophobic products, but less than that of BIOVECTOR S, which imitates the natural functioning of the skin, and has a dual mechanism: it slows the insensible water loss, and captures several times its own weight in water, thereby maintaining humidity at the skin's surface.

## 2. GPS-M

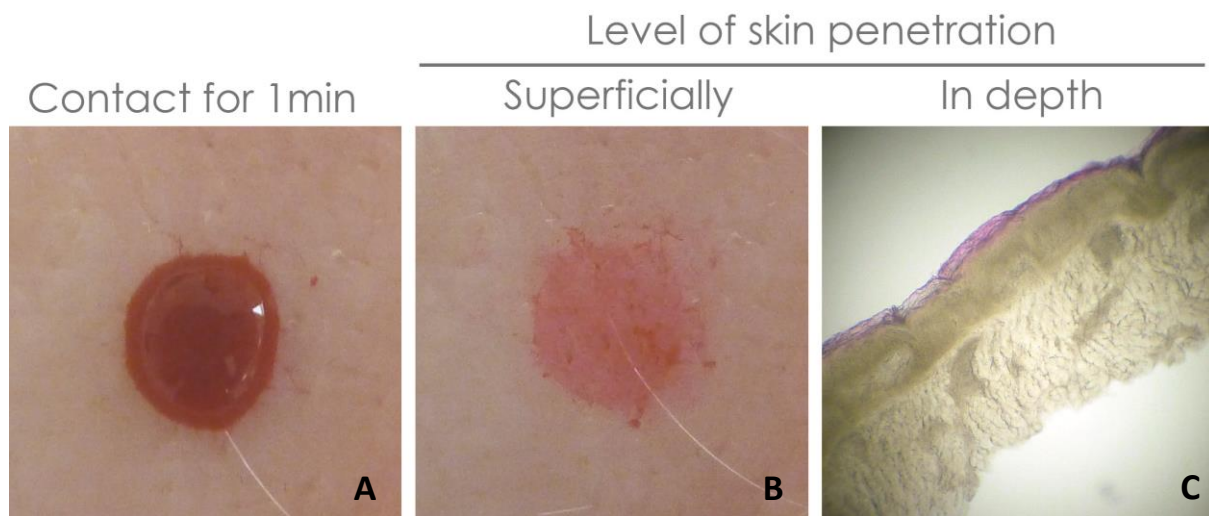
### 2.1. EFFICIENCY IN TERMS OF MOLECULAR DIFFUSION (EX-VIVO).

A skin penetration test was carried out with median biovector, as follows.

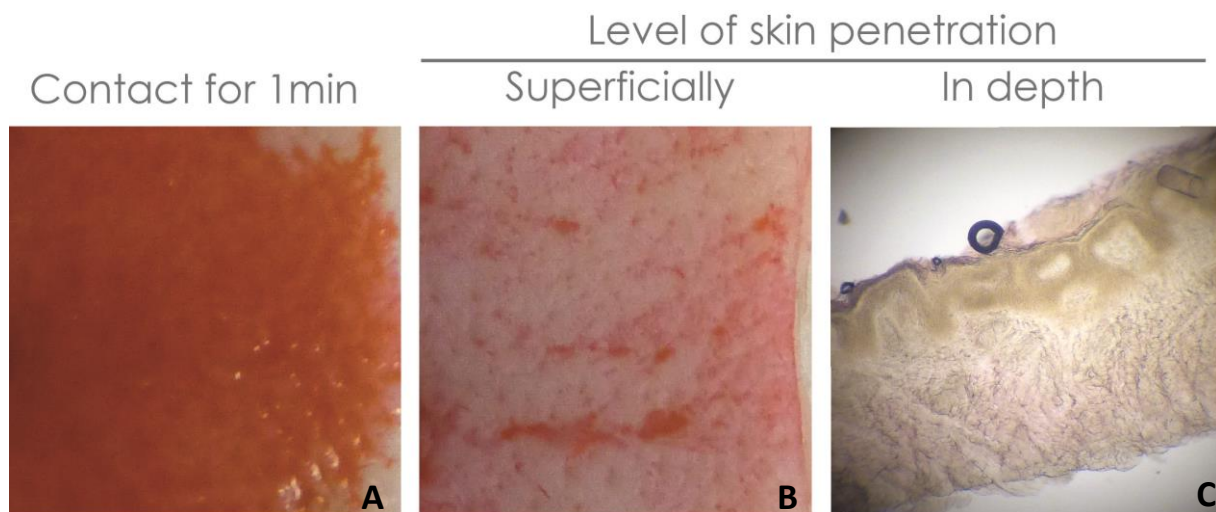
In order to demonstrate the invention's efficiency in terms of the diffusion of active ingredients through the various layers of the skin:

-we applied (Fig. 2) or did not apply (Fig.1) the median biovector to the surface of the skin of a pig's ear.

**FIGURE 1**



**FIGURE 2**



-one drop of red dye (eosine) was deposited and left in contact with the skin of a pig's ear for one minute (Figs. 1A and 2A).

-after one minute of contact, the skin was wiped without rinsing, using absorbent paper (Figs. 1B and 2B).

-a 50 µm histological section was made using a vibratome on the central part of the zone on which the drop of dye was deposited. The section was then observed under the microscope (magnification x100), in order to visualize the degree of penetration and/or the bioavailability of the product at the level of the tissue (Figs. 1C and 2C).

Without the median biovector, the dye penetrates the skin superficially (Fig. 1C), whereas application using the median biovector promoted homogeneous diffusion of the active ingredient into the middle layers of the skin (Fig. 2C).

## 2.2. EFFICIENCY IN TERMS OF MOLECULAR DIFFUSION (IN-VIVO).

A skin penetration test was carried out on human skin with the median biovector, as described in the following.

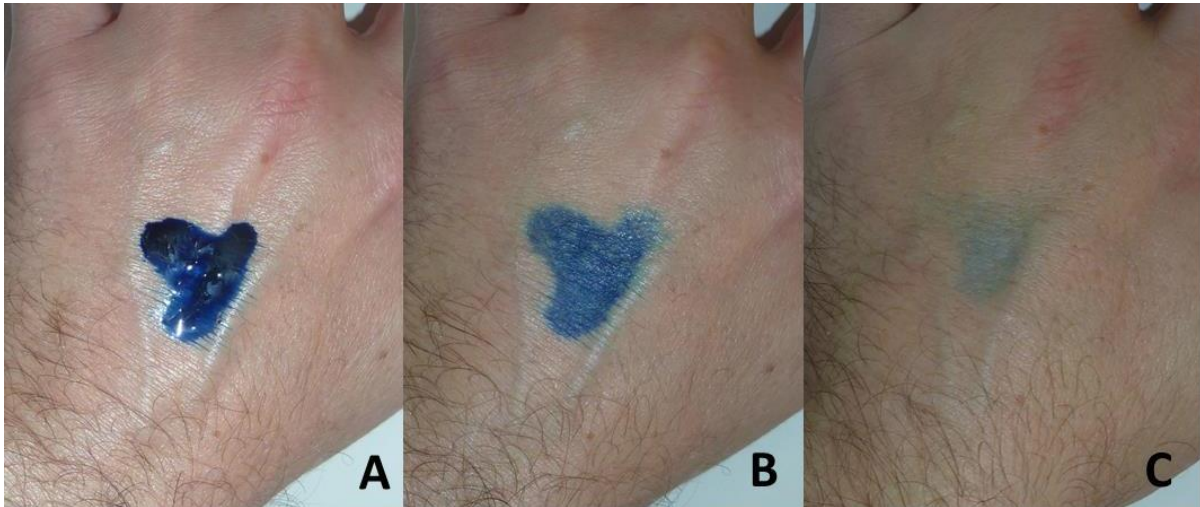
In order to demonstrate the efficiency of the median biovector on the diffusion of active ingredients through the different layers of the skin, the biovector was colored with blue dye.

The dye combination (Fig. 4A) or the dye alone (Fig. 3A) were applied to the skin. One minute following application, the products were wiped without rinsing. The dye has slightly penetrated the skin in the case of the control (Fig. 3B), whereas with the combination of the invention it has not only diffused at the skin's surface, but has also penetrated it more significantly (intensity of the color) (Fig. 4B).

**FIGURE 3 CONTROL TEST**



**FIGURE 4 TREATED WITH BIOVECTOR M**



In both cases, rinsing with detergent and water was carried out for 10 seconds. In the control case, the dye has been totally washed away, whereas with the combination of the invention, the dye remains present in the middle layers of the skin.

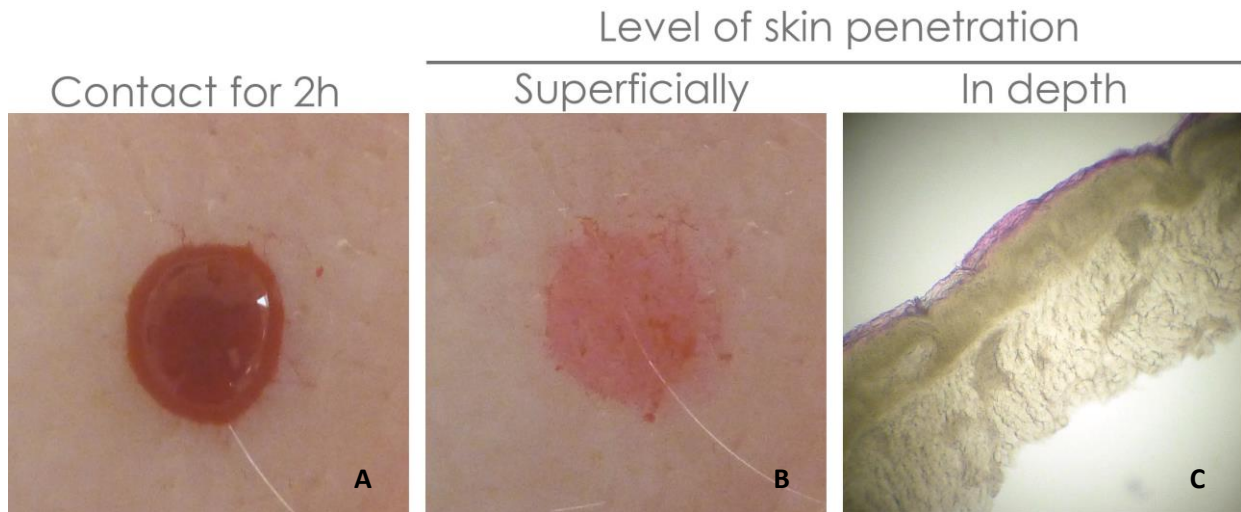
### 3. GPS-P

#### 3.1. EFFICIENCY IN TERMS OF MOLECULAR DIFFUSION (EX-VIVO).

A skin penetration test was carried out with the deep biovector, as described in the following. In order to demonstrate the efficiency of the invention on the diffusion of active ingredients through the different layers of the skin, we:

- applied (Fig. 2) or did not apply (Fig. 1) the deep biovector to the surface of the skin of a pig's ear.
- one drop of red dye (eosine) was deposited and left in contact with the skin of a pig's ear for two hours (Figs. 1A and 2A).
- following two hours of contact, the skin was wiped without rinsing, using absorbent paper (Figs. 1B and 2B).
- a 50 µm histological section was made using a vibratome on the central part of the zone on which the drop of dye was deposited. The section was then observed under the microscope (magnification x100), in order to visualize the degree of penetration and/or the bioavailability of the product at the level of the tissue (Figs. 1C and 2C).

**FIGURE 1**



**FIGURE 2**



Without the deep biovector, the dye penetrated the skin superficially (Fig. 1C), whereas the application using the biovector promoted homogeneous diffusion of the active ingredient into the deep layers of the skin (Fig. 2C). More intense staining can also be seen at the level of the skin appendages (hair shaft), showing that the adnexal passage is promoted by this biovector, thus allowing the deeper layers of the dermis to be more easily reached.

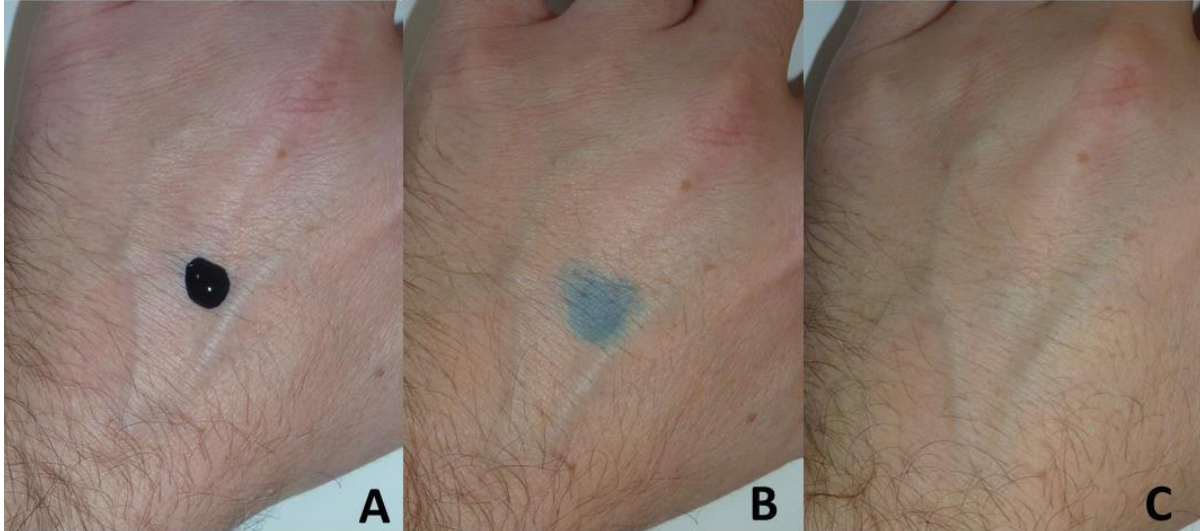
### 3.2. EFFICIENCY IN TERMS OF MOLECULAR DIFFUSION (IN-VIVO).

A skin penetration test was carried out with the deep biovector on human skin, as described in the following.

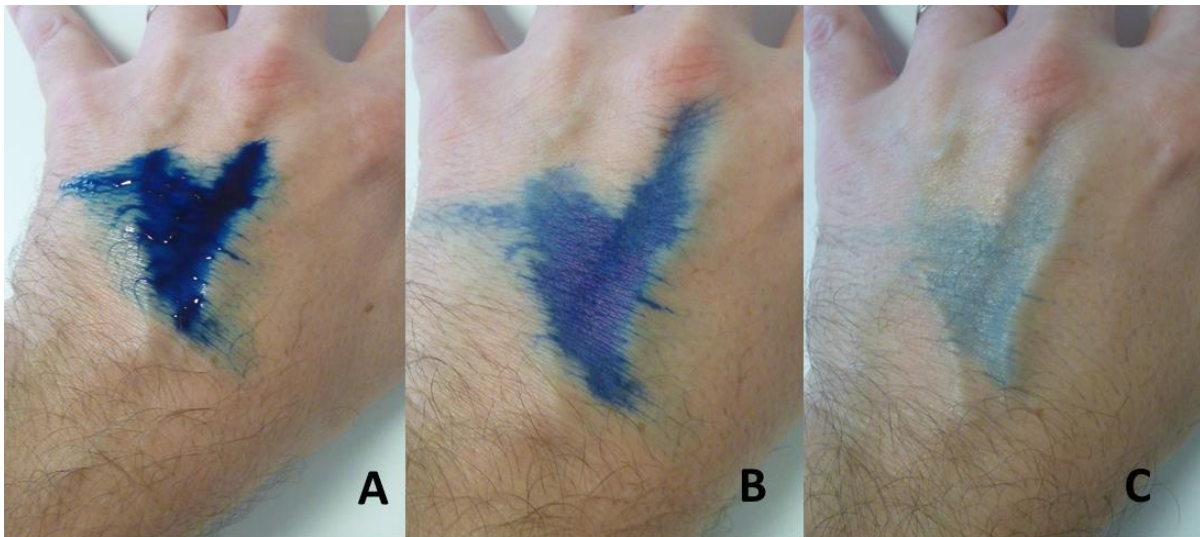
In order to demonstrate the efficiency of the deep biovector on the diffusion of active ingredients through the different layers of the skin, the biovector was colored with blue dye.

The dye combination (Fig. 4A) or the dye alone (Fig. 3A) were applied to the skin. One minute following application, the products were wiped without rinsing.

**FIGURE 3 CONTROL TEST**



**FIGURE 4 TREATED WITH BIOVECTOR P**



The dye has slightly penetrated the skin in the case of the control (Fig. 3B), whereas with the (biovector-dye) combination it has not only diffused at the skin's surface, but also penetrated it more significantly (intensity of the color) (Fig. 4B). In both cases, rinsing with detergent and water was carried out for 20 seconds. In the control case, the dye has been totally washed away, whereas with the combination of the invention, the dye remains present in the deep layers of the skin.

## PART V: CONCLUSION.

### 1. GPS-S

Thanks to its specific molecular combination, BIOVECTOR S has numerous useful cosmetic properties:

- it ensures that the molecules of the active ingredient are maintained at the surface of the epithelia, allowing the concentration of the active ingredient to be limited, passive diffusion outside the targeted zone to be avoided, and the results to be enhanced.
- it forms a cutaneous “barrier” at the skin’s surface, allowing it to be protected from external aggressions by reducing the penetration of molecules / agents present in the environment (pollution, bacteria, fungi, ...) and by preventing the penetration of potentially toxic molecules present in cosmetic products (UV filters, nanoparticles), perfumes (allergens, ...), ....
- it associates itself with the hydrolipidic film, thereby limiting the insensible water loss.
- it restructures the epithelia having a defective organization and/or cellular cohesion, in the hair in particular.

To summarize, the following applications could be envisaged for BIOVECTOR S:

- inducing a barrier effect by opposing the penetration of any exogenous substance through the skin.
- inducing a protective effect with respect to physical, chemical, mechanical or thermal alterations.
- reducing the bioavailability of substances added to the preparation.
- reducing the toxic risk.
- reducing the volatility of certain volatile and/or odorous substances, thereby increasing the olfactory persistence of these substances.
- keeping the substances at the level of the contact zone and increasing the persistence effect.
- promoting the penetration of substances applied to the skin prior to combination.
- softening skin, appendages or hide.
- reducing the insensible loss of water originating from the dermis and reaching the upper layers of the epidermis.

## 2. GPS-M

Thanks to its specific molecular combination, BIOVECTOR M has the properties of a biovector for all molecules, for which the cosmetic, dermatological and/or pharmaceutical interest lies in the middle layers of the skin.

BIOVECTOR M:

- Promotes the diffusion of active molecules through the corneal layer of the epidermis.
- Allows the constraints associated with the physico-chemical characteristics of the molecule to be partially avoided, by transporting it to the relevant active site.
- Ensures that the active molecules are maintained in the middle part of the skin (lower zone of the epidermis and upper zone of the dermis), allowing the concentration of the active ingredient to be limited, passive diffusion outside the targeted zone to be avoided, and the results to be enhanced.

By virtue of the properties of the median biovector, it will be possible for BIOVECTOR M to be associated with any substance, which is naturally bioavailable or not, designed to act on the cells of the epidermis and/or the basal lamina, with the aim of stimulating, protecting, and/or inhibiting these. Of interest, although not exclusively, are the Langerhan cells, fibroblasts, melanocytes, keratinocytes and the extracellular matrix.

Possible applications could include the following:

- BIOVECTOR M allows and/or promotes the skin penetration of active molecules of high molecular weight. Molecules of high molecular weight have a low or inexistent skin penetration (hyaluronic acid, collagen, vitamin D, ...).
- Thanks to its biovector properties, BIOVECTOR M will be able to act on skin hydration by promoting, for example, the penetration and diffusion of hygroscopic molecules, the penetration of which is often difficult as a consequence, in particular, of the high molecular weight of said substances. The inclusion of BIOVECTOR M into a cosmetic formula facilitates the penetration and fixation of hygroscopic molecules, which store water and contribute to a reduction in evaporation.
- Thanks to its biovector properties, BIOVECTOR M will be able to act on the skin's immune system, by promoting, for example, the diffusion of molecules activating the Langerhan cells. The Langerhan cells, situated at the level of the dermis, constitute the skin's immune system. As the skin has the greatest surface area in contact with

potentially pathogenic elements in the external environment (viruses, bacteria, toxins, ...), it is essential for it to have an efficient defense system. However, since these cells are embedded in the epidermis, it will be advantageous to associate all types of agents with BIOVECTOR M, making it possible to pass through the corneal layer, and allowing the Langerhan cells to be reached, with the aim of stimulating them and/or initiating their action.

- Thanks to its biovector properties, BIOVECTOR M will be able to act on the physico-chemical characteristics of the extracellular matrix of the dermis by acting, for example, on the fibroblasts. The fibroblasts are the main cells of the dermis. They are specialized in the synthesis of two types of protein fibers: collagen fibers and elastin fibers of which the extracellular matrix is composed. These fibers give the skin its resistance to tension and traction, as well as its elastic properties. Their stimulation requires, with the help of BIOVECTOR M, overcoming the relative impermeability of the corneal layer or the stratum corneum.
- Thanks to its biovector properties, BIOVECTOR M will be able to act on skin pigmentation, by acting for example on the melanocytes. Pigmentation of the skin is a complex process, which begins with the synthesis of melanin by the melanocytes. In humans, the entire melanocyte population is located in the hair follicles and in the basal layer of the epidermis. In addition to its aesthetic aspect, the main role of melanin is to protect the skin against the harmful effects of UV light. The association with BIOVECTOR M, of molecules stimulating or inhibiting the production of melanin, will increase the bioavailability of the associated substances and will limit the loss of active ingredients through the various cellular layers.
- Thanks to its biovector properties, BIOVECTOR M will also be able to act on an anaesthetizing effect. BIOVECTOR M's ability to reach the dermal papillae containing the nerve fibers, which penetrate the basal lamina to innervate the epidermis (connection to nerve corpuscles in the dermis, which act as tactile mechanoreceptors), is useful in cosmetics, in particular in the context of epilation or medical situations for an improved and/or faster action of anaesthetizing patches and/or topical skin applications used in aesthetic medicine.

### 3. GPS-P

Thanks to its specific molecular combination, BIOVECTOR P has the properties of a biovector for all molecules, for which the cosmetic, dermatological and/or pharmaceutical interest lies in the deep layers of the skin.

BIOVECTOR P:

- Promotes the diffusion of active molecules through the corneal layer of the epidermis (intercellular and adnexal paths).
- Allows the constraints associated with the physico-chemical characteristics of the molecule to be partially avoided, by transporting it to the relevant active site.
- Ensures that the active molecules are kept in the deep part of the skin (lower zone of the dermis and hypodermis), allowing the concentration of the active ingredient to be limited, passive diffusion outside the targeted zone to be avoided, and the results to be enhanced.

By virtue of the properties of the deep biovector, it will be possible for BIOVECTOR P to be associated with any substance which is naturally bioavailable or not, designed to act on the cells of the dermis and the hypodermis (or other cells through a systemic action), with the aim of stimulating, protecting, and/or inhibiting these. Of interest are, although not exclusively, the fibroblasts, adipocytes, endothelial cells, and muscular cells, ... .

Possible applications could include the following:

- BIOVECTOR P allows and/or promotes the bioavailability of pharmaceutical molecules. In pharmacology, bioavailability is defined by the speed of absorption and the quantity of medication absorbed. That represents the fraction of a dose reaching the bloodstream without any change. Bioavailability must be taken into account when calculating dosages for non-intravenous routes of administration.
- Thanks to its biovector properties, BIOVECTOR P can transport active ingredients such as lipolytics to the adipocytes of the hypodermis, which are often difficult to penetrate since they have a low bioavailability, or caffeine for slimming effects. The hypodermis is a granular tissue made up from specific cells grouped in clusters and able to store lipids: the adipocytes. Active ingredients with slimming effects will be able to act by reducing the storage of lipids (lipogenesis) or by promoting the use of lipids (lipolysis). Lipogenesis is a synthesis phase in the energy reserve process of the adipocytes. It is initiated in dietary triglycerides, fatty acids or dietary carbohydrates. Lipolysis is the lipid degradation reaction which produces energy. The

complex lipids, mainly triglycerides, are firstly hydrolyzed into fatty acids, which are then transformed according to one of the following routes: 1]- beta-oxidation: this takes place in the mitochondria and produces Acetyl-Coenzyme A at the level of the Lynen helix. In the presence of oxygen, Acetyl Coenzyme A is integrated into the Krebs cycle and produces CO<sub>2</sub> and energy in the form of ATP, as well as reduced coenzymes. Beta-oxidation can also take place in the peroxisome, producing ATP and heat. 2]- Cetogenesis: this takes place in the liver. During fasting (> 18h) the Krebs cycle no longer functions, since it is deviated for the production of glucose by means of neoglucogenesis. The beta-oxidation of fatty acids, which are the only energetic substrates in the liver, then provokes the accumulation of Acetyl coenzyme A in the hepatic cells. The Acetyl coenzyme A is thus transformed to ketones, which are exported by the blood and then used as a substitute for glucose (heart/nerve cells).

- Thanks to its biovector capacities, and by targeting the deep layers of the skin, BIOVECTOR P is able to increase the efficiency of active molecules, which regulate microcirculation in the skin. When the epidermis is not vascularized, the dermis continues to receive blood through the capillaries. The vessels must be attained at the level of the fatty lobules, in order to ensure the presence of a systemic path and/or an action on the general system. Skin circulation allows essential elements to be transported for the correct functioning of the cells: nutrients, oxygen, water, antibodies, ... . Any dysfunction of skin microcirculation can result in redness, itchiness, blotchy skin, a surface inflammation, lack of tone, loss in density and/or elasticity at the level of the dermis, and cellulitis in the hypodermis.
- Thanks to its biovector capacities for certain molecules, and by targeting the deep layers of the skin, BIOVECTOR P carries all active molecules towards, thereby stimulating or regulating, the lymphatic system. Water retention can be defined as the excessive accumulation of water in all or part of the organism. Water retention occurs when the organism accumulates more water than it eliminates, for hormonal and/or circulatory reasons. Thanks to the facilitation of hydric movements, detoxification of the skin is promoted. The association of the present invention with all molecules designed to act topically on the blood's circulation, and/or on the lymphatic circulation, will promote and/or regulate hydric circulation and detoxification of the skin.

In addition, BIOVECTOR P will be highly beneficial for formulators, by improving its touch, spreadability, penetration, and all sensorial characteristics, even in extreme contexts of ointments or a high hydrocarbon load.

## PARTIE VI : MARKETING



POUR NE PLUS PERDRE L'EFFICACITÉ DE VOTRE SOIN, **CHOISISSEZ UN SOIN AVEC GPS\* INTÉGRÉ.**



GUIDAGE DE SURFACE    GUIDAGE MOYEN    GUIDAGE EN PROFONDEUR

**\*GUIDAGE PERFORMANCE SOIN**  
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**!INNOVI**  
 Une vision nouvelle dans les sciences de vivant



Un amincissant  
qui va droit au but ?  
Je suis pour !

**ICI,** découvrez  
le 1<sup>er</sup> soin anti-ride  
avec GPS\* intégré.

\*Guidage Performance Soin



**SAFRAL**  
Donne du sens à la beauté.



Une crème  
anti-ride qui sait  
où elle va ?  
Je suis pour !



**ICI,** découvrez  
le 1<sup>er</sup> soin anti-ride  
avec GPS\* intégré.

\*Guidage Performance Soin



**SAFRAL**  
Donne du sens à la beauté.

Des soins qui perdent leur  
efficacité en chemin ?  
Nous sommes contre !

**ICI**, découvrez les  
1<sup>ers</sup> soins avec GPS\* intégré.

\*Guidage Performance Soin



**SAFRAL**  
Donne du sens à la beauté.



france

LABORATOIRES DE RECHERCHE ET D'EXPERIMENTATION

**STUDY/TEST ITEM REFERENCES**

**: B14 0167 / 14-0366**

**SPONSOR**

**: INNOVI**  
La Sablière  
RN 21  
47390 LAYRAC  
France

**TEST ITEM**

**: GPS-M Batch 1802145**

**ASSESSMENT OF THE IRRITANT POTENTIAL OF A TEST ITEM AFTER APPLICATION TO THE  
EMBRYONIC HEN'S EGG CHORIOALLANTOIC MEMBRANE**

**-HET-CAM-**

**Final report**

**Bordeaux, March 20, 2014**

**11 pages in this report**



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**EVALUATION DU POTENTIEL IRRITANT D'UN ITEM D'ESSAI PAR APPLICATION  
SUR LA MEMBRANE CHORIO-ALLANTOÏDIENNE DE L'OEUF DE POULE EMBRYONNE  
- HET-CAM -**

**ASSESSMENT OF THE IRRITANT POTENTIAL OF A TEST ITEM AFTER APPLICATION  
TO THE EMBRYONIC HEN'S EGG CHORIOALLANTOIC MEMBRANE  
- HET-CAM -**

**RESUME/ SUMMARY**

• **PRINCIPE DE L'ETUDE/ PRINCIPLE OF THE STUDY**

L'étude a été basée sur l'observation, par une personne qualifiée, des effets irritants (hyperhémie, hémorragie, coagulation) pouvant survenir dans les cinq minutes suivant le dépôt d'un élément d'essai sur la membrane chorioallantoïdienne (MCA) d'œufs de poule embryonnés au dixième jour d'incubation.

Le potentiel irritant a été scoré selon une échelle allant de 0 à 21. L'élément d'essai a été classé dans l'une des catégories définies en fonction du score moyen obtenu.

*The study was based on the observation, by a trained person, of the irritant effects (hyperhemia, haemorrhage and coagulation) occurring during the five minutes after application of test item to the chorioallantoic membrane (CAM) of embryonic hen's eggs on the tenth day of incubation.*

*The irritant potential was scored according to a scale from 0 to 21. The test item was classified in one of the categories defined according to the mean score obtained.*

<b>Score moyen/ Mean Score (Scm/ MSc)</b>	<b>Classification/ Classification</b>
Scm/MSc < 1	Pratiquement non irritant/ Practically non irritant
$1 \leq \text{Scm/MSc} < 5$	Faiblement irritant/ Slightly irritant
$5 \leq \text{Scm/MSc} < 9$	Modérément irritant/ Moderately irritant
$\text{Scm/MSc} \geq 9$	Irritant/ Irritant

• **DATE DE DEBUT ET DE FIN D'EXPERIMENTATION / EXPERIMENTAL STARTING DATE AND EXPERIMENTAL COMPLETION DATE** : 24 février 2014 / February 24, 2014

• **RESULTATS/ RESULTS**:

<b>Élément d'essai Test item</b>	<b>Concentration testée Tested concentration</b>	<b>Score moyen sur 4 œufs ±écart type Mean score on 4 eggs ±standard deviation</b>	<b>Classification Classification</b>	<b>Comparaison par rapport à des éléments d'essai appartenant à la même catégorie Comparison with test items belonging to the same category</b>
<b>GPS-M Batch 1802145</b>	Diluée à 3 % dans l'eau p.p.i. / Diluted at 3 % with water for injection	0 ± 0	pratiquement non irritant / practically non irritant	pas de comparaison disponible / no available comparison

## **I . AIM AND PRINCIPLE OF THE STUDY**

The Sponsor requested to assess semi-quantitatively the irritant potential of the test item, **GPS-M Batch 1802145** by an alternative method to animal testing: application to the embryonic hen's egg chorioallantoic membrane (Het-Cam).

The study was based on the observation, by a trained person, of the irritant effects (hyperhemia, haemorrhage and coagulation) that could occur within the five minutes after application of the test item to the embryonic hen's egg choriollantoic membrane (CAM), on the tenth day of incubation.

The technique used was an adaptation from the one described by Luepke N.P. and Kemper F.H. (The Het-Cam test: "An alternative to the Draize eye test". Food Chem. Toxicol. 1986, 24, n° 6/7, 495-496).

This method followed the text of the Order of November 29, 1996 published in the Official Journal of the French Republic of December 26, 1996, except for:

- the weight of the eggs (between 40 and 75g instead of between 50 and 65 g).
- the destruction of eggs by quick cooling (enclosure at -20°C) instead of an injection of pentobarbital.

These deviations to the text of the Official Journal of the French Republic of December 26, 1996 had no impact on the validity of the study.

This method is an alternative to animal testing forming part of a range of tests which aim at assessing the ocular irritant potential of the test items (in particular the ones based on surfactants).

## **II. TEST FACILITY AND TECHNICAL STAFF**

### **II.1. Test facility and technical staff**

#### **Evic France –Bio Department**

122 rue Croix de Seguey  
33000 Bordeaux

Phone : 05 56 95 59 95

Study Director: Sarah JULIENNE

Responsible technicians: Mathieu LE MERRER, Sarah JULIENNE

### **II.2. Approval of the Test Facility**

The study was entirely performed in the premises of the Bio Technical Department of Evic France Company, in Bordeaux.

The Evic Bio test facility was recognized in accordance with the principles of Good Laboratory Practices by the ANSM (order of August 10, 2004 published in the Official Journal of the French Republic (OJRF) of September 18, 2004 and order of March 14, 2000 published in the OJRF of March 23, 2000) and the GIPC (decree No 2006-1523 of December 4, 2006 published in the Official Journal of the French Republic of December 6, 2006).

## **III. DATA COLLECTION**

All the data collected during the study were recorded by the technician responsible for the study, on the documents reserved to that effect.

Each page of these documents was initialled and dated by the technician responsible for the study. The missing data and the corrections were justified, initialled and dated.

At the end of the study, the work document was filed with the final report and they will be kept for 3 years in the filing room validated by the Quality Assurance department of the Test Facility.

This filing room is located at the service provider's premises. The service provider is not subject to GLP scope but guarantees the quality and the integrity of the documents and study data and is submitted to regular audits by the Quality Assurance department.

At the end of this period, the Test Facility will define with the sponsor, the carrying out of the filing, the restitution of the data or their destruction.

#### **IV. QUALITY ASSURANCE**

The Quality Assurance Unit checked by regular audits, the respect of the study plan and of the working procedures relating to this type of study.

Audits relating to the Test Facility were carried out every year. These audits enabled to check that the Test Facility met the requirements of the Authorities.

Controls were performed by the different persons involved in the test since the end of the experimentation until the sending of the final report. These different controls were formalized through the filling up of a self-control sheet present in the test file.

All the experimental data and the final report were audited by the Assurance Quality Personnel.

#### **V. STUDY PLAN MODIFICATIONS**

In this study as no modification of the standard study plan referenced PEG.HC.01/13(11)-ANSM was noticed no amendment was established.

#### **VI. TEST ITEM**

##### **VI.1. Reference of the test item**

Evic France identification	: 14-0366
Receipt date	: February 19, 2014
Identification	: GPS-M
Batch N°	: 1802145
Category of the test item	: cosmetic ingredient
Description/Aspect	: off-white cream
Storage conditions	: ambient temperature and out of the light in a room especially fitted out for that effect
Particularity	: None
Quantity provided by the sponsor	: 1 plastic jar of 30 ml
Expiration date	: February, 2016
Other information supplied	: formula

Stable under the storage and test conditions.

##### **VI.2. Test item filing**

The test item or a remaining part of the test item was filed in the samples library of the Test Facility and will be kept for a period of 6 months.

#### **VII. TEST SYSTEM**

Embryonic White Leghorn Hen's eggs, graded between 40 and 75 g supplied by: INRA Station off aviary pathology and parantology SPF poultry and rabbit area - 37380 NOUZILLY

### **VII.1. Eggs receipt**

On receipt, the eggs were observed one by one. Eggs cracked or broken were eliminated. Afterwards eggs were weighted. Eggs out of the graded range were eliminated.

The eggs were identified by a batch number (week/year) which was given to the eggs at receipt. Each selected egg is identified individually by the day of incubation and put into a thermostated oven at  $12 \pm 1^\circ\text{C}$  out of the light, for at least 24 hours before to put them into the incubator.

### **VII.2. Putting into incubator**

Eggs were put into an incubator, under controlled conditions of temperature ( $37.8^\circ\text{C} \pm 1^\circ\text{C}$ ) and relative hygrometry (50 to 60 %).

During their 10-day incubation period, eggs were placed, in an incubator with oscillating plates in upright position (air pocket upwards).

## **VIII. DATES OF THE STUDY**

Study starting date (signature of the study plan specific complement): February 24, 2014

Experimental starting date: February 24, 2014

Experimental completion date: February 24, 2014

Study completion date (signature of the final report): March 20, 2014

## **IX. TEST PROCEDURE**

### **IX.1. Preparation of the test item**

The test item was tested diluted at 3 % with water for injection.

The process of dilution was performed extemporaneously in weight/weight with water for injection (Cooper batch 19FC28GA) with a precision balance.

Test item appearance after dilution : nacreous white liquid.

The dilution of the test item was brought at  $37 \pm 1^\circ\text{C}$  before use.

### **IX.2. Experimental chronology**

#### **IX.2.1. Preparation of the eggs**

The different steps of the study were performed quickly under a constant lighting, which did not give out too much heat in order to avoid the withering of the chorioallantoic membrane.

On the 10<sup>th</sup> day of incubation, eggs were taken out of the incubator one by one and were candled with a lamp. The defective eggs (image which did not correspond to the expected stage of development) were eliminated and the selected eggs were put on the holder, "air pocket" upwards.

The shell of each selected egg was drilled (with a lanceolate needle), opened and cut up (with scissors with blunt ends or pliers) at air pocket level and until the limits of the shell membrane.

Then the whole surface of the shell membrane was moistened with a 0.9 % sodium chloride solution, warmed up at  $37^\circ\text{C}$  (bain-marie). Then, tilting of the egg eliminated the excess of 0.9 % sodium chloride solution and the shell membrane was removed delicately with pliers in order to uncover the underlying CAM.

Any egg whose chorioallantoic membrane was damaged (tear, presence of haemorrhage and any other lesion) was immediately rejected.

#### IX.2.2. Application of the dilution of the test item

The dilution of the test item was tested on 4 eggs.

300 µl was deposited on the CAM, with a micropipette (P1000).

Immediately after application, the chronometer was set off.

#### IX.2.3. Readings

After 20 seconds' contact, the CAM was rinsed with 5 ml of sodium chloride isotonic solution (kept at 37°C in bain-marie), with a syringe avoiding any brutal projection.

Tilting of the egg eliminated the rinse liquid.

The possible phenomena of irritation were observed during 5 minutes according to the process described in the following paragraph. The accurate time of each phenomenon appearance was noted.

The 20 seconds' contact was included in the 5 minutes' observation.

At the end of the study, embryos were destroyed by quick cooling (enclosure at -20°C).

#### IX.2.4. Process of reading

The observations taken into account for the notation of the test item were performed visually, under lamp.

The observed phenomena (hyperhemia, haemorrhage, coagulation) were not retained according to their intensity but to their presence: it is an all or nothing response.

The time was noted as soon as the appearance of each phenomenon.

The phenomena observed were defined as follows:

##### **Hyperhemia**

Phenomenon observed: capillaries that were not visible before the addition of the product become visible whereas the visible capillaries dilate and become redder. This phenomenon can also affect the vessels of higher diameter.

##### **Haemorrhage**

Phenomenon observed: release of blood escaping from vessels and/or capillaries, taking different appearances, and in particular in "cauliflower", in layer, in diffuse veil, in dotted (blood selectively escapes from different places of the membrane).

It is worth noting that:

- the haemorrhage can have a short-lived characteristic; however it must be taken into account,
- the observation, during the first 30 seconds, of a massive haemorrhage requires taking into account the hidden hyperhemia.

##### **Coagulation (opacity and/or thrombosis)**

###### **Opacity:**

Phenomenon observed: appearance on the whole or part of the membrane, either of an opalescent veil possibly evolving to opacity or of a direct opacity.

It is necessary to check that this phenomenon is not related to the chemico-physical behaviour of the product in aqueous medium (for example formation of a colloid, precipitate;...).

### Thrombosis

Phenomenon observed: rupture of blood flow in the vessels resulting in a segmented appearance (alternation between constrictions and more or less dark turgescence areas).

It is worth noting that the observations must not take into account the changes that occurred on capillaries.

### IX.3. Expression and interpretation of the results

The observed phenomena were quantified according to the table below, in accordance with the rate of appearance:

Phenomenon	Times		
	$t \leq 30 \text{ s}$	$30 \text{ s} < t \leq 2 \text{ min}$	$2 \text{ min} < t \leq 5 \text{ min}$
Hyperhemias	5	3	1
Haemorrhage	7	5	3
Coagulation	9	7	5

Each phenomenon observed was only counted once, when it occurred.

The score for each egg was the sum of the hyperhemias, haemorrhage and coagulation notes. The notation of the test item was the arithmetical mean of the scores obtained on 4 eggs, rounded up to one decimal (maximal notation = 21).

The following scale gave the irritant potential of the test item on the chorioallantoic membrane:

Mean Score (MSc)	Classification
$MSc < 1$	Practically non irritant
$1 \leq MSc < 5$	Slightly irritant
$5 \leq MSc < 9$	Moderately irritant
$MSc \geq 9$	Irritant

## X. CONTROL OF THE TEST SYSTEM, OPERATIVE CONDITIONS AND EXPERIMENTERS

### X.1. Negative control

This control which allowed checking the quality of the eggs, was realized with 0.9 % NaCl solution (batch 19FC12GA) pre-warmed at  $37 \pm 1^\circ\text{C}$  before eggs treatment.

The control complied when the given score was between 0.0 and 3.0.

### X.2. Positive control

The quality of the test system, operative conditions and experimenters was checked using a reference.

This blind control was performed through a standard curve of 0.05 %, 0.4 % and 3.2 % lauryl sulfobetaine solutions (Sigma, batch 1421027V) in water for injectable preparations (Cooper, batch 19FC28GA) pre-warmed at  $37 \pm 1^\circ\text{C}$ .

The control complied when:

- the 0.05 % concentration gave a score between 0.0 and 5.0
- the 0.4 % concentration gave a score between 10.5 and 12.5
- the 3.2 % concentration gave a score between 17.0 and 21.0

The scores of the positive and negative controls were indicated in the table below.

**X.3. Controls results**

Batch	Control	Conc. (%)	Date	Scoring	Mean Score	±	Standard Deviation
Week 09/14	NaCl 0.9 %	As supplied	February 24, 2014	On 2 eggs (negative control)	0.0	±	0.0
01/14	Lauryl sulfobetaine	3.2 %	January 02, 2014	On 6 eggs	20.3	±	1.0
01/14	Lauryl sulfobetaine	0.4 %	January 02, 2014	On 6 eggs	12.0	±	0.0
01/14	Lauryl sulfobetaine	0.05 %	January 02, 2014	On 6 eggs	0.0	±	0.0

**XI. RESULTS**

Eggs	Hyperhemia Quotation according to time ≤ 30 sec = 5 ≤ 2 min = 3 ≤ 5 min = 1			Haemorrhage Quotation according to time ≤ 30 sec = 7 ≤ 2 min = 5 ≤ 5 min = 3		Coagulation Quotation according to time ≤ 30 sec = 9 ≤ 2 min = 7 ≤ 5 min = 5			Score by egg
	Observed/Hidden	Times (s)	note	Times (s)	note	Opacity/Thrombosis	Times (s)	note	
1	/	/	0	/	0	/	/	0	0
2	/	/	0	/	0	/	/	0	0
3	/	/	0	/	0	/	/	0	0
4	/	/	0	/	0	/	/	0	0
Mean			0.0		0.0			0.0	0.0
S.D			0.0		0.0			0.0	0.0

**XII. CONCLUSION**

According to the defined scale, the test item **GPS-M Batch 1802145** tested diluted at 3 % with water for injection, was considered as **practically non irritant** towards the chorioallantoic membrane of embryonic hen's eggs.

Test item	Tested concentration	Mean score on 4 Eggs ± Standard Deviation	Classification
<b>GPS-M Batch 1802145</b>	Diluted at 3 % with water for injection	0 ± 0	practically non irritant

The response obtained for the test item cannot be compared, due to a lack of historical data in that category of product (cosmetic ingredient).

### XIII. STUDY DIRECTOR ATTESTATION

The study **B14 0167** intended to assess the irritant potential of the test item **GPS-M Batch 1802145**, after application to the embryonic hen's egg chorioallantoic membrane (method Het-Cam).

The technique used was an adaptation from the one described by Luepke N.P. and Kemper F.H. (The Het-Cam test: "An alternative to the Draize eye test". Food Chem. Toxicol. 1986, 24, n° 6/7, 495-496).

This method was described in the French order of November 29, 1996, published in the Official Journal of the French Republic of December 26, 1996.

The sponsor asked this study to be carried out according to the principles of the Good Laboratory Practice of in reference to the texts below:

- OECD Principles on Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM (98) 17 and all subsequent consensus documents,
- Directive 2004/10/EC of the European Parliament and of the Council of 11 February 2004 on the harmonization of laws, regulations and administrative provisions relating to the application of the Principles of Good Laboratory Practice and the verification of their applications for tests on chemical substances (OJ No. L50 of 20.2.2004),
- Order of August 10th, 2004 taken to be the application of the article L. 5131-5 of the code of the public health relating to the Good Laboratory Practice for cosmetics product (Official journal n° 218 of September 18th, 2004), Ministry of Health and social welfare system.

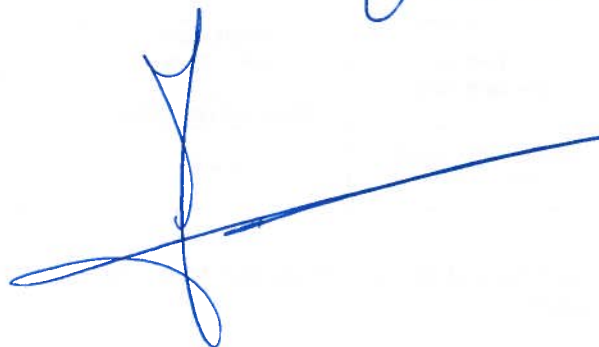
I undersigned, **Sarah JULIENNE**, declare that this study is in accordance with the above-mentioned texts of the Good Laboratory Practice.

Sarah JULIENNE

Date :

Study director :

March 20, 2014



**XIV. QUALITY ASSURANCE ATTESTATION**

I the undersigned, **Florence PROD'HOMME**, for the Quality Assurance Unit, declare that :

- The Quality Assurance Unit ensured the strict application of the Test Facility's current procedures

Audits about the Test Facility were performed once a year. These audits allowed checking whether the Test Facility met the requirements of the authorities or not.

Study-based audits (documentation and process appropriate to this type of study) were carried out; the result of Quality Assurance evaluations was documented and reported to the Study Director and Management as follows:

Dates of audits	Audited phases	Dates of audit report transmission to study Director	Dates of audit report transmission to Management
February 17, 2014	Test conduct*	February 24, 2014	February 24, 2014
March 20, 2014	Final report	March 20, 2014	March 21, 2014

\* Audit conduct on a study of the same type.

The final report fully and accurately reflects the procedures and the data generated during the study.

Only the paper version of the final report is legally acceptable.

---

Florence PROD'HOMME

Date : 20.03.14

Quality Assurance :





**EVIC**france

LABORATOIRES DE RECHERCHE ET D'EXPERIMENTATION

**STUDY/TEST ITEM REFERENCES**

**: B14 0168 / 14-0367**

**SPONSOR**

**: INNOVI**  
La Sablière  
RN 21  
47390 LAYRAC  
France

**TEST ITEM**

**: GPS-P Batch 1802146**

**ASSESSMENT OF THE IRRITANT POTENTIAL OF A TEST ITEM AFTER APPLICATION TO THE  
EMBRYONIC HEN'S EGG CHORIOALLANTOIC MEMBRANE**

**-HET-CAM-**

**Final report**

**Bordeaux, March 20, 2014**

**11 pages in this report**



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**EVALUATION DU POTENTIEL IRRITANT D'UN ITEM D'ESSAI PAR APPLICATION  
SUR LA MEMBRANE CHORIO-ALLANTOÏDIENNE DE L'OEUF DE POULE EMBRYONNE  
- HET-CAM -**

**ASSESSMENT OF THE IRRITANT POTENTIAL OF A TEST ITEM AFTER APPLICATION  
TO THE EMBRYONIC HEN'S EGG CHORIOALLANTOIC MEMBRANE  
- HET-CAM -**

**RESUME / SUMMARY**

• **PRINCIPE DE L'ETUDE / PRINCIPLE OF THE STUDY**

L'étude a été basée sur l'observation, par une personne qualifiée, des effets irritants (hyperhémie, hémorragie, coagulation) pouvant survenir dans les cinq minutes suivant le dépôt d'un élément d'essai sur la membrane chorioallantoïdienne (MCA) d'œufs de poule embryonnés au dixième jour d'incubation.

Le potentiel irritant a été scoré selon une échelle allant de 0 à 21. L'élément d'essai a été classé dans l'une des catégories définies en fonction du score moyen obtenu.

*The study was based on the observation, by a trained person, of the irritant effects (hyperhemia, haemorrhage and coagulation) occurring during the five minutes after application of test item to the chorioallantoic membrane (CAM) of embryonic hen's eggs on the tenth day of incubation.*

*The irritant potential was scored according to a scale from 0 to 21. The test item was classified in one of the categories defined according to the mean score obtained.*

<b>Score moyen / Mean Score (Scm / MSc)</b>	<b>Classification / Classification</b>
Scm / MSc < 1	Pratiquement non irritant / Practically non irritant
$1 \leq \text{Scm} / \text{MSc} < 5$	Faiblement irritant / Slightly irritant
$5 \leq \text{Scm} / \text{MSc} < 9$	Modérément irritant / Moderately irritant
$\text{Scm} / \text{MSc} \geq 9$	Irritant / Irritant

• **DATE DE DEBUT ET DE FIN D'EXPERIMENTATION / EXPERIMENTAL STARTING DATE  
AND EXPERIMENTAL COMPLETION DATE : 24 février 2014 / February 24, 2014**

• **RESULTATS / RESULTS :**

<b>Élément d'essai Test item</b>	<b>Concentration testée Tested concentration</b>	<b>Score moyen sur 4 œufs ±écart type Mean score on 4 eggs ±standard deviation</b>	<b>Classification Classification</b>	<b>Comparaison par rapport à des éléments d'essai appartenant à la même catégorie Comparison with test items belonging to the same category</b>
<b>GPS-P Batch 1802146</b>	Diluée à 3 % dans l'eau p.p.i. / Diluted at 3 % with water for injection	1.5 ± 1.7	Faiblement irritant / Slightly irritant	pas de comparaison disponible / no available comparison

## **I . AIM AND PRINCIPLE OF THE STUDY**

The Sponsor requested to assess semi-quantitatively the irritant potential of the test item, **GPS-P Batch 1802146** by an alternative method to animal testing: application to the embryonic hen's egg chorioallantoic membrane (Het-Cam).

The study was based on the observation, by a trained person, of the irritant effects (hyperhemia, haemorrhage and coagulation) that could occur within the five minutes after application of the test item to the embryonic hen's egg chorioallantoic membrane (CAM), on the tenth day of incubation.

The technique used was an adaptation from the one described by Luepke N.P. and Kemper F.H. (The Het-Cam test: "An alternative to the Draize eye test". Food Chem. Toxicol. 1986, 24, n° 6/7, 495-496).

This method followed the text of the Order of November 29, 1996 published in the Official Journal of the French Republic of December 26, 1996, except for:

- the weight of the eggs (between 40 and 75g instead of between 50 and 65 g).
- the destruction of eggs by quick cooling (enclosure at -20°C) instead of an injection of pentobarbital.

These deviations to the text of the Official Journal of the French Republic of December 26, 1996 had no impact on the validity of the study.

This method is an alternative to animal testing forming part of a range of tests which aim at assessing the ocular irritant potential of the test items (in particular the ones based on surfactants).

## **II. TEST FACILITY AND TECHNICAL STAFF**

### **II.1. Test facility and technical staff**

#### **Evic France –Bio Department**

122 rue Croix de Seguey  
33000 Bordeaux

Phone : 05 56 95 59 95

Study Director: Sarah JULIENNE

Responsible technicians: Mathieu LE MERRER, Sarah JULIENNE

### **II.2. Approval of the Test Facility**

The study was entirely performed in the premises of the Bio Technical Department of Evic France Company, in Bordeaux.

The Evic Bio test facility was recognized in accordance with the principles of Good Laboratory Practices by the ANSM (order of August 10, 2004 published in the Official Journal of the French Republic (OJRF) of September 18, 2004 and order of March 14, 2000 published in the OJRF of March 23, 2000) and the GIPC (decree No 2006-1523 of December 4, 2006 published in the Official Journal of the French Republic of December 6, 2006).

## **III. DATA COLLECTION**

All the data collected during the study were recorded by the technician responsible for the study, on the documents reserved to that effect.

Each page of these documents was initialled and dated by the technician responsible for the study. The missing data and the corrections were justified, initialled and dated.

At the end of the study, the work document was filed with the final report and they will be kept for 3 years in the filing room validated by the Quality Assurance department of the Test Facility.

This filing room is located at the service provider's premises. The service provider is not subject to GLP scope but guarantees the quality and the integrity of the documents and study data and is submitted to regular audits by the Quality Assurance department.

At the end of this period, the Test Facility will define with the sponsor, the carrying out of the filing, the restitution of the data or their destruction.

#### **IV. QUALITY ASSURANCE**

The Quality Assurance Unit checked by regular audits, the respect of the study plan and of the working procedures relating to this type of study.

Audits relating to the Test Facility were carried out every year. These audits enabled to check that the Test Facility met the requirements of the Authorities.

Controls were performed by the different persons involved in the test since the end of the experimentation until the sending of the final report. These different controls were formalized through the filling up of a self-control sheet present in the test file.

All the experimental data and the final report were audited by the Assurance Quality Personnel.

#### **V. STUDY PLAN MODIFICATIONS**

In this study as no modification of the standard study plan referenced PEG.HC.01/13(11)-ANSM was noticed no amendment was established.

#### **VI. TEST ITEM**

##### **VI.1. Reference of the test item**

Evic France identification	: 14-0367
Receipt date	: February 19, 2014
Identification	: GPS-P
Batch N°	: 1802146
Category of the test item	: cosmetic ingredient
Description/Aspect	: clear yellow liquid
Storage conditions	: ambient temperature and out of the light in a room especially fitted out for that effect
Particularity	: 46 % alcohol
Quantity provided by the sponsor	: 1 plastic jar of 30 ml
Expiration date	: February, 2016
Other information supplied	: formula

Stable under the storage and test conditions.

##### **VI.2. Test item filing**

The test item or a remaining part of the test item was filed in the samples library of the Test Facility and will be kept for a period of 6 months.

#### **VII. TEST SYSTEM**

Embryonic White Leghorn Hen's eggs, graded between 40 and 75 g supplied by: INRA Station off aviary pathology and parantology SPF poultry and rabbit area - 37380 NOUZILLY

### **VII.1. Eggs receipt**

On receipt, the eggs were observed one by one. Eggs cracked or broken were eliminated. Afterwards eggs were weighted. Eggs out of the graded range were eliminated.

The eggs were identified by a batch number (week/year) which was given to the eggs at receipt. Each selected egg is identified individually by the day of incubation and put into a thermostated oven at  $12 \pm 1^\circ\text{C}$  out of the light, for at least 24 hours before to put them into the incubator.

### **VII.2. Putting into incubator**

Eggs were put into an incubator, under controlled conditions of temperature ( $37.8^\circ\text{C} \pm 1^\circ\text{C}$ ) and relative hygrometry (50 to 60 %).

During their 10-day incubation period, eggs were placed, in an incubator with oscillating plates in upright position (air pocket upwards).

## **VIII. DATES OF THE STUDY**

Study starting date (signature of the study plan specific complement): February 24, 2014

Experimental starting date: February 24, 2014

Experimental completion date: February 24, 2014

Study completion date (signature of the final report): March 20, 2014

## **IX. TEST PROCEDURE**

### **IX.1. Preparation of the test item**

The test item was tested diluted at 3 % with water for injection.

The process of dilution was performed extemporaneously in weight/weight with water for injection (Cooper batch 19FC28GA) with a precision balance.

Test item appearance after dilution : colourless transparent liquid.

The dilution of the test item was tested at room temperature.

### **IX.2. Experimental chronology**

#### **IX.2.1. Preparation of the eggs**

The different steps of the study were performed quickly under a constant lighting, which did not give out too much heat in order to avoid the withering of the chorioallantoic membrane.

On the 10<sup>th</sup> day of incubation, eggs were taken out of the incubator one by one and were candled with a lamp. The defective eggs (image which did not correspond to the expected stage of development) were eliminated and the selected eggs were put on the holder, "air pocket" upwards.

The shell of each selected egg was drilled (with a lanceolate needle), opened and cut up (with scissors with blunt ends or pliers) at air pocket level and until the limits of the shell membrane.

Then the whole surface of the shell membrane was moistened with a 0.9 % sodium chloride solution, warmed up at  $37^\circ\text{C}$  (bain-marie). Then, tilting of the egg eliminated the excess of 0.9 % sodium chloride solution and the shell membrane was removed delicately with pliers in order to uncover the underlying CAM.

Any egg whose chorioallantoic membrane was damaged (tear, presence of haemorrhage and any other lesion) was immediately rejected.

#### IX.2.2. Application of the dilution of the test item

The dilution of the test item was tested on 4 eggs.

300 µl was deposited on the CAM, with a micropipette (P1000).

Immediately after application, the chronometer was set off.

#### IX.2.3. Readings

After 20 seconds' contact, the CAM was rinsed with 5 ml of sodium chloride isotonic solution (kept at 37°C in bain-marie), with a syringe avoiding any brutal projection.

Tilting of the egg eliminated the rinse liquid.

The possible phenomena of irritation were observed during 5 minutes according to the process described in the following paragraph. The accurate time of each phenomenon appearance was noted.

The 20 seconds' contact was included in the 5 minutes' observation.

At the end of the study, embryos were destroyed by quick cooling (enclosure at -20°C).

#### IX.2.4. Process of reading

The observations taken into account for the notation of the test item were performed visually, under lamp.

The observed phenomena (hyperhemia, haemorrhage, coagulation) were not retained according to their intensity but to their presence: it is an all or nothing response.

The time was noted as soon as the appearance of each phenomenon.

The phenomena observed were defined as follows:

##### **Hyperhemia**

Phenomenon observed: capillaries that were not visible before the addition of the product become visible whereas the visible capillaries dilate and become redder. This phenomenon can also affect the vessels of higher diameter.

##### **Haemorrhage**

Phenomenon observed: release of blood escaping from vessels and/or capillaries, taking different appearances, and in particular in "cauliflower", in layer, in diffuse veil, in dotted (blood selectively escapes from different places of the membrane).

It is worth noting that:

- the haemorrhage can have a short-lived characteristic; however it must be taken into account,
- the observation, during the first 30 seconds, of a massive haemorrhage requires taking into account the hidden hyperhemia.

##### **Coagulation (opacity and/or thrombosis)**

###### **Opacity:**

Phenomenon observed: appearance on the whole or part of the membrane, either of an opalescent veil possibly evolving to opacity or of a direct opacity.

It is necessary to check that this phenomenon is not related to the chemico-physical behaviour of the product in aqueous medium (for example formation of a colloid, precipitate;...).

### Thrombosis

Phenomenon observed: rupture of blood flow in the vessels resulting in a segmented appearance (alternation between constrictions and more or less dark turgescient areas).

It is worth noting that the observations must not take into account the changes that occurred on capillaries.

### IX.3. Expression and interpretation of the results

The observed phenomena were quantified according to the table below, in accordance with the rate of appearance:

Phenomenon	Times		
	$t \leq 30 \text{ s}$	$30 \text{ s} < t \leq 2 \text{ min}$	$2 \text{ min} < t \leq 5 \text{ min}$
Hyperhemia	5	3	1
Haemorrhage	7	5	3
Coagulation	9	7	5

Each phenomenon observed was only counted once, when it occurred.

The score for each egg was the sum of the hyperhemia, haemorrhage and coagulation notes. The notation of the test item was the arithmetical mean of the scores obtained on 4 eggs, rounded up to one decimal (maximal notation = 21).

The following scale gave the irritant potential of the test item on the chorioallantoic membrane:

Mean Score (MSc)	Classification
$MSc < 1$	Practically non irritant
$1 \leq MSc < 5$	Slightly irritant
$5 \leq MSc < 9$	Moderately irritant
$MSc \geq 9$	Irritant

## X. CONTROL OF THE TEST SYSTEM, OPERATIVE CONDITIONS AND EXPERIMENTERS

### X.1. Negative control

This control which allowed checking the quality of the eggs, was realized with 0.9 % NaCl solution (batch 19FC12GA) pre-warmed at  $37 \pm 1^\circ\text{C}$  before eggs treatment.

The control complied when the given score was between 0.0 and 3.0.

### X.2. Positive control

The quality of the test system, operative conditions and experimenters was checked using a reference.

This blind control was performed through a standard curve of 0.05 %, 0.4 % and 3.2 % lauryl sulfobetaine solutions (Sigma, batch 1421027V) in water for injectable preparations (Cooper, batch 19FC28GA) pre-warmed at  $37 \pm 1^\circ\text{C}$ .

The control complied when:

- the 0.05 % concentration gave a score between 0.0 and 5.0
- the 0.4 % concentration gave a score between 10.5 and 12.5
- the 3.2 % concentration gave a score between 17.0 and 21.0

The scores of the positive and negative controls were indicated in the table below.

### X.3. Controls results

Batch	Control	Conc. (%)	Date	Scorage	Mean Score	±	Standard Deviation
Week 09/14	NaCl 0.9 %	As supplied	February 24, 2014	On 2 eggs (negative control)	0.0	±	0.0
01/14	Lauryl sulfobetaine	3.2 %	January 02, 2014	On 6 eggs	20.3	±	1.0
01/14	Lauryl sulfobetaine	0.4 %	January 02, 2014	On 6 eggs	12.0	±	0.0
01/14	Lauryl sulfobetaine	0.05 %	January 02, 2014	On 6 eggs	0.0	±	0.0

## XI. RESULTS

Eggs	Hyperhemia Quotation according to time ≤ 30 sec = 5 ≤ 2 min = 3 ≤ 5 min = 1			Haemorrhage Quotation according to time ≤ 30 sec = 7 ≤ 2 min = 5 ≤ 5 min = 3		Coagulation Quotation according to time ≤ 30 sec = 9 ≤ 2 min = 7 ≤ 5 min = 5			Score by egg
	Observed/ Hidden	Times (s)	note	Times (s)	note	Opacity/ Thrombosis	Times (s)	note	
1	/	/	0	/	0	/	/	0	0
2	/	/	0	/	0	/	/	0	0
3	Observed	33	3	/	0	/	/	0	3
4	Observed	32	3	/	0	/	/	0	3
Mean			1.5		0.0			0.0	1.5
S.D			1.7		0.0			0.0	1.7

## XII. CONCLUSION

According to the defined scale, the test item **GPS-P Batch 1802146** tested diluted at 3 % with water for injection, was considered as **slightly irritant** towards the chorioallantoic membrane of embryonic hen's eggs.

Test item	Tested concentration	Mean score on 4 Eggs ± Standard Deviation	Classification
<b>GPS-P Batch 1802146</b>	Diluted at 3 % with water for injection	1.5 ± 1.7	slightly irritant

The response obtained for the test item cannot be compared, due to a lack of historical data in that category of product (cosmetic ingredient).

### XIII. STUDY DIRECTOR ATTESTATION

The study **B14 0168** intended to assess the irritant potential of the test item **GPS-P Batch 1802146**, after application to the embryonic hen's egg chorioallantoic membrane (method Het-Cam).

The technique used was an adaptation from the one described by Luepke N.P. and Kemper F.H. (The Het-Cam test: "An alternative to the Draize eye test". Food Chem. Toxicol. 1986, 24, n° 6/7, 495-496).

This method was described in the French order of November 29, 1996, published in the Official Journal of the French Republic of December 26, 1996.

The sponsor asked this study to be carried out according to the principles of the Good Laboratory Practice of in reference to the texts below:

- OECD Principles on Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM (98) 17 and all subsequent consensus documents,
- Directive 2004/10/EC of the European Parliament and of the Council of 11 February 2004 on the harmonization of laws, regulations and administrative provisions relating to the application of the Principles of Good Laboratory Practice and the verification of their applications for tests on chemical substances (OJ No. L50 of 20.2.2004),
- Order of August 10th, 2004 taken to be the application of the article L. 5131-5 of the code of the public health relating to the Good Laboratory Practice for cosmetics product (Official journal n° 218 of September 18th, 2004), Ministry of Health and social welfare system.

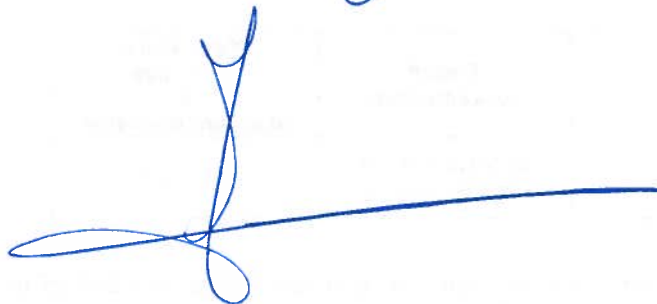
I undersigned, **Sarah JULIENNE**, declare that this study is in accordance with the above-mentioned texts of the Good Laboratory Practice.

Sarah JULIENNE

Date :

Study director :

*Sarah 20, 2014*



**XIV. QUALITY ASSURANCE ATTESTATION**

I the undersigned, **Florence PROD'HOMME**, for the Quality Assurance Unit, declare that :

- The Quality Assurance Unit ensured the strict application of the Test Facility's current procedures

Audits about the Test Facility were performed once a year. These audits allowed checking whether the Test Facility met the requirements of the authorities or not.

Study-based audits (documentation and process appropriate to this type of study) were carried out; the result of Quality Assurance evaluations was documented and reported to the Study Director and Management as follows:

Dates of audits	Audited phases	Dates of audit report transmission to study Director	Dates of audit report transmission to Management
February 17, 2014	Test conduct*	February 24, 2014	February 24, 2014
March 20, 2014	Final report	March 20, 2014	March 21, 2014

\* Audit conduct on a study of the same type.

The final report fully and accurately reflects the procedures and the data generated during the study.

Only the paper version of the final report is legally acceptable.

---

Florence PROD'HOMME

Date : 20.03.14

Quality Assurance :





**EVIC** france

LABORATOIRES DE RECHERCHE ET D'EXPERIMENTATION

**STUDY/TEST ITEM REFERENCES** : **B14 0166 / 14-0365**

**SPONSOR** : INNOVI  
La Sablière  
RN 21  
47390 LAYRAC  
France

**TEST ITEM** : GPS-S Batch 1802144

**ASSESSMENT OF THE IRRITANT POTENTIAL OF A TEST ITEM AFTER APPLICATION TO THE EMBRYONIC HEN'S EGG CHORIOALLANTOIC MEMBRANE**

**-HET-CAM-**

**Final report**

**Bordeaux, March 20, 2014**

**11 pages in this report**



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**EVALUATION DU POTENTIEL IRRITANT D'UN ITEM D'ESSAI PAR APPLICATION  
SUR LA MEMBRANE CHORIO-ALLANTOÏDIENNE DE L'OEUF DE POULE EMBRYONNE  
- HET-CAM -**

**ASSESSMENT OF THE IRRITANT POTENTIAL OF A TEST ITEM AFTER APPLICATION  
TO THE EMBRYONIC HEN'S EGG CHORIOALLANTOIC MEMBRANE  
- HET-CAM -**

**RESUME/ SUMMARY**

• **PRINCIPE DE L'ETUDE/ PRINCIPLE OF THE STUDY**

L'étude a été basée sur l'observation, par une personne qualifiée, des effets irritants (hyperhémie, hémorragie, coagulation) pouvant survenir dans les cinq minutes suivant le dépôt d'un élément d'essai sur la membrane chorioallantoïdienne (MCA) d'œufs de poule embryonnés au dixième jour d'incubation.

Le potentiel irritant a été scoré selon une échelle allant de 0 à 21. L'élément d'essai a été classé dans l'une des catégories définies en fonction du score moyen obtenu.

*The study was based on the observation, by a trained person, of the irritant effects (hyperhemia, haemorrhage and coagulation) occurring during the five minutes after application of test item to the chorioallantoic membrane (CAM) of embryonic hen's eggs on the tenth day of incubation.*

*The irritant potential was scored according to a scale from 0 to 21. The test item was classified in one of the categories defined according to the mean score obtained.*

<b>Score moyen/ Mean Score (Scm/ MSc)</b>	<b>Classification/ Classification</b>
Scm/MSc < 1	Pratiquement non irritant/ Practically non irritant
1 ≤ Scm/MSc < 5	Faiblement irritant/ Slightly irritant
5 ≤ Scm/MSc < 9	Modérément irritant/ Moderately irritant
Scm/MSc ≥ 9	Irritant/ Irritant

• **DATE DE DEBUT ET DE FIN D'EXPERIMENTATION / EXPERIMENTAL STARTING DATE  
AND EXPERIMENTAL COMPLETION DATE : 24 février 2014 / February 24, 2014**

• **RESULTATS/ RESULTS:**

<b>Elément d'essai Test item</b>	<b>Concentration testée Tested concentration</b>	<b>Score moyen sur 4 œufs ± écart type Mean score on 4 eggs ± standard deviation</b>	<b>Classification Classification</b>	<b>Comparaison par rapport à des éléments d'essai appartenant à la même catégorie Comparison with test items belonging to the same category</b>
<b>GPS-S Batch 1802144</b>	Diluée à 3 % dans l'eau p.p.i. / Diluted at 3 % with water for injection	0 ± 0	pratiquement non irritant / practically non irritant	pas de comparaison disponible / no available comparison

## **I . AIM AND PRINCIPLE OF THE STUDY**

The Sponsor requested to assess semi-quantitatively the irritant potential of the test item, **GPS-S Batch 1802144** by an alternative method to animal testing: application to the embryonic hen's egg chorioallantoic membrane (Het-Cam).

The study was based on the observation, by a trained person, of the irritant effects (hyperhemia, haemorrhage and coagulation) that could occur within the five minutes after application of the test item to the embryonic hen's egg chorioallantoic membrane (CAM), on the tenth day of incubation.

The technique used was an adaptation from the one described by Luepke N.P. and Kemper F.H. (The Het-Cam test: "An alternative to the Draize eye test". Food Chem. Toxicol. 1986, 24, n° 6/7, 495-496).

This method followed the text of the Order of November 29, 1996 published in the Official Journal of the French Republic of December 26, 1996, except for:

- the weight of the eggs (between 40 and 75g instead of between 50 and 65 g).
- the destruction of eggs by quick cooling (enclosure at -20°C) instead of an injection of pentobarbital.

These deviations to the text of the Official Journal of the French Republic of December 26, 1996 had no impact on the validity of the study.

This method is an alternative to animal testing forming part of a range of tests which aim at assessing the ocular irritant potential of the test items (in particular the ones based on surfactants).

## **II. TEST FACILITY AND TECHNICAL STAFF**

### **II.1. Test facility and technical staff**

#### **Evic France –Bio Department**

122 rue Croix de Seguey  
33000 Bordeaux

Phone : 05 56 95 59 95

Study Director: Sarah JULIENNE

Responsible technicians: Mathieu LE MERRER, Sarah JULIENNE

### **II.2. Approval of the Test Facility**

The study was entirely performed in the premises of the Bio Technical Department of Evic France Company, in Bordeaux.

The Evic Bio test facility was recognized in accordance with the principles of Good Laboratory Practices by the ANSM (order of August 10, 2004 published in the Official Journal of the French Republic (OJRF) of September 18, 2004 and order of March 14, 2000 published in the OJRF of March 23, 2000) and the GIPC (decree No 2006-1523 of December 4, 2006 published in the Official Journal of the French Republic of December 6, 2006).

## **III. DATA COLLECTION**

All the data collected during the study were recorded by the technician responsible for the study, on the documents reserved to that effect.

Each page of these documents was initialled and dated by the technician responsible for the study. The missing data and the corrections were justified, initialled and dated.

At the end of the study, the work document was filed with the final report and they will be kept for 3 years in the filing room validated by the Quality Assurance department of the Test Facility.

This filing room is located at the service provider's premises. The service provider is not subject to GLP scope but guarantees the quality and the integrity of the documents and study data and is submitted to regular audits by the Quality Assurance department.

At the end of this period, the Test Facility will define with the sponsor, the carrying out of the filing, the restitution of the data or their destruction.

#### **IV. QUALITY ASSURANCE**

The Quality Assurance Unit checked by regular audits, the respect of the study plan and of the working procedures relating to this type of study.

Audits relating to the Test Facility were carried out every year. These audits enabled to check that the Test Facility met the requirements of the Authorities.

Controls were performed by the different persons involved in the test since the end of the experimentation until the sending of the final report. These different controls were formalized through the filling up of a self-control sheet present in the test file.

All the experimental data and the final report were audited by the Assurance Quality Personnel.

#### **V. STUDY PLAN MODIFICATIONS**

In this study as no modification of the standard study plan referenced PEG.HC.01/13(11)-ANSM was noticed no amendment was established.

#### **VI. TEST ITEM**

##### **VI.1. Reference of the test item**

Evic France identification	: 14-0365
Receipt date	: February 19, 2014
Identification	: GPS-S
Batch N°	: 1802144
Category of the test item	: cosmetic ingredient
Description/Aspect	: clear beige to white cream
Storage conditions	: ambient temperature and out of the light in a room especially fitted out for that effect
Particularity	: None
Quantity provided by the sponsor	: 1 plastic jar of 30 ml
Expiration date	: February, 2016
Other information supplied	: formula

Stable under the storage and test conditions.

##### **VI.2. Test item filing**

The test item or a remaining part of the test item was filed in the samples library of the Test Facility and will be kept for a period of 6 months.

#### **VII. TEST SYSTEM**

Embryonic White Leghorn Hen's eggs, graded between 40 and 75 g supplied by: INRA Station off aviary pathology and parantology SPF poultry and rabbit area - 37380 NOUZILLY

### **VII.1. Eggs receipt**

On receipt, the eggs were observed one by one. Eggs cracked or broken were eliminated. Afterwards eggs were weighted. Eggs out of the graded range were eliminated.

The eggs were identified by a batch number (week/year) which was given to the eggs at receipt. Each selected egg is identified individually by the day of incubation and put into a thermostated oven at  $12 \pm 1^\circ\text{C}$  out of the light, for at least 24 hours before to put them into the incubator.

### **VII.2. Putting into incubator**

Eggs were put into an incubator, under controlled conditions of temperature ( $37.8^\circ\text{C} \pm 1^\circ\text{C}$ ) and relative hygrometry (50 to 60 %).

During their 10-day incubation period, eggs were placed, in an incubator with oscillating plates in upright position (air pocket upwards).

## **VIII. DATES OF THE STUDY**

Study starting date (signature of the study plan specific complement): February 24, 2014

Experimental starting date: February 24, 2014

Experimental completion date: February 24, 2014

Study completion date (signature of the final report): March 20, 2014

## **IX. TEST PROCEDURE**

### **IX.1. Preparation of the test item**

The test item was tested diluted at 3 % with water for injection.

The process of dilution was performed extemporaneously in weight/weight with water for injection (Cooper batch 19FC28GA) with a precision balance.

Test item appearance after dilution : white opaque liquid

The dilution of the test item was brought at  $37 \pm 1^\circ\text{C}$  before use..

### **IX.2. Experimental chronology**

#### **IX.2.1. Preparation of the eggs**

The different steps of the study were performed quickly under a constant lighting, which did not give out too much heat in order to avoid the withering of the chorioallantoic membrane.

On the 10<sup>th</sup> day of incubation, eggs were taken out of the incubator one by one and were candled with a lamp. The defective eggs (image which did not correspond to the expected stage of development) were eliminated and the selected eggs were put on the holder, "air pocket" upwards.

The shell of each selected egg was drilled (with a lanceolate needle), opened and cut up (with scissors with blunt ends or pliers) at air pocket level and until the limits of the shell membrane.

Then the whole surface of the shell membrane was moistened with a 0.9 % sodium chloride solution, warmed up at  $37^\circ\text{C}$  (bain-marie). Then, tilting of the egg eliminated the excess of 0.9 % sodium chloride solution and the shell membrane was removed delicately with pliers in order to uncover the underlying CAM.

Any egg whose chorioallantoic membrane was damaged (tear, presence of haemorrhage and any other lesion) was immediately rejected.

#### IX.2.2. Application of the dilution of the test item

The dilution of the test item was tested on 4 eggs.

300 µl was deposited on the CAM, with a micropipette (P1000).

Immediately after application, the chronometer was set off.

#### IX.2.3. Readings

After 20 seconds' contact, the CAM was rinsed with 5 ml of sodium chloride isotonic solution (kept at 37°C in bain-marie), with a syringe avoiding any brutal projection.

Tilting of the egg eliminated the rinse liquid.

The possible phenomena of irritation were observed during 5 minutes according to the process described in the following paragraph. The accurate time of each phenomenon appearance was noted.

The 20 seconds' contact was included in the 5 minutes' observation.

At the end of the study, embryos were destroyed by quick cooling (enclosure at -20°C).

#### IX.2.4. Process of reading

The observations taken into account for the notation of the test item were performed visually, under lamp.

The observed phenomena (hyperhemia, haemorrhage, coagulation) were not retained according to their intensity but to their presence: it is an all or nothing response.

The time was noted as soon as the appearance of each phenomenon.

The phenomena observed were defined as follows:

##### **Hyperhemia**

Phenomenon observed: capillaries that were not visible before the addition of the product become visible whereas the visible capillaries dilate and become redder. This phenomenon can also affect the vessels of higher diameter.

##### **Haemorrhage**

Phenomenon observed: release of blood escaping from vessels and/or capillaries, taking different appearances, and in particular in "cauliflower", in layer, in diffuse veil, in dotted (blood selectively escapes from different places of the membrane).

It is worth noting that:

- the haemorrhage can have a short-lived characteristic; however it must be taken into account,
- the observation, during the first 30 seconds, of a massive haemorrhage requires taking into account the hidden hyperhemia.

##### **Coagulation (opacity and/or thrombosis)**

###### **Opacity:**

Phenomenon observed: appearance on the whole or part of the membrane, either of an opalescent veil possibly evolving to opacity or of a direct opacity.

It is necessary to check that this phenomenon is not related to the chemico-physical behaviour of the product in aqueous medium (for example formation of a colloid, precipitate;...).

### Thrombosis

Phenomenon observed: rupture of blood flow in the vessels resulting in a segmented appearance (alternation between constrictions and more or less dark turgescient areas).

It is worth noting that the observations must not take into account the changes that occurred on capillaries.

### IX.3. Expression and interpretation of the results

The observed phenomena were quantified according to the table below, in accordance with the rate of appearance:

Phenomenon	Times		
	$t \leq 30 \text{ s}$	$30 \text{ s} < t \leq 2 \text{ min}$	$2 \text{ min} < t \leq 5 \text{ min}$
Hyperhemia	5	3	1
Haemorrhage	7	5	3
Coagulation	9	7	5

Each phenomenon observed was only counted once, when it occurred.

The score for each egg was the sum of the hyperhemia, haemorrhage and coagulation notes. The notation of the test item was the arithmetical mean of the scores obtained on 4 eggs, rounded up to one decimal (maximal notation = 21).

The following scale gave the irritant potential of the test item on the chorioallantoic membrane:

Mean Score (MSc)	Classification
$MSc < 1$	Practically non irritant
$1 \leq MSc < 5$	Slightly irritant
$5 \leq MSc < 9$	Moderately irritant
$MSc \geq 9$	Irritant

## X. CONTROL OF THE TEST SYSTEM, OPERATIVE CONDITIONS AND EXPERIMENTERS

### X.1. Negative control

This control which allowed checking the quality of the eggs, was realized with 0.9 % NaCl solution (batch 19FC12GA) pre-warmed at  $37 \pm 1^\circ\text{C}$  before eggs treatment.

The control complied when the given score was between 0.0 and 3.0.

### X.2. Positive control

The quality of the test system, operative conditions and experimenters was checked using a reference.

This blind control was performed through a standard curve of 0.05 %, 0.4 % and 3.2 % lauryl sulfobetaine solutions (Sigma, batch 1421027V) in water for injectable preparations (Cooper, batch 19FC28GA) pre-warmed at  $37 \pm 1^\circ\text{C}$ .

The control complied when:

- the 0.05 % concentration gave a score between 0.0 and 5.0
- the 0.4 % concentration gave a score between 10.5 and 12.5
- the 3.2 % concentration gave a score between 17.0 and 21.0

The scores of the positive and negative controls were indicated in the table below.

Batch	Control	Conc. (%)	Date	Scoring	Mean Score	±	Standard Deviation
Week 09/14	NaCl 0.9 %	As supplied	February 24, 2014	On 2 eggs (negative control)	0.0	±	0.0
01/14	Lauryl sulfobetaine	3.2 %	January 02, 2014	On 6 eggs	20.3	±	1.0
01/14	Lauryl sulfobetaine	0.4 %	January 02, 2014	On 6 eggs	12.0	±	0.0
01/14	Lauryl sulfobetaine	0.05 %	January 02, 2014	On 6 eggs	0.0	±	0.0

## XI. RESULTS

Eggs	Hyperhemia Quotation according to time ≤ 30 sec = 5 ≤ 2 min = 3 ≤ 5 min = 1			Haemorrhage Quotation according to time ≤ 30 sec = 7 ≤ 2 min = 5 ≤ 5 min = 3		Coagulation Quotation according to time ≤ 30 sec = 9 ≤ 2 min = 7 ≤ 5 min = 5			Score by egg
	Observed/Hidden	Times (s)	note	Times (s)	note	Opacity/Thrombosis	Times (s)	note	
1	/	/	0	/	0	/	/	0	0
2	/	/	0	/	0	/	/	0	0
3	/	/	0	/	0	/	/	0	0
4	/	/	0	/	0	/	/	0	0
Mean			0.0		0.0			0.0	0.0
S.D			0.0		0.0			0.0	0.0

## XII. CONCLUSION

According to the defined scale, the test item **GPS-S Batch 1802144** tested diluted at 3 % with water for injection, was considered as **practically non irritant** towards the chorioallantoic membrane of embryonic hen's eggs.

Test item	Tested concentration	Mean score on 4 Eggs ± Standard Deviation	Classification
<b>GPS-S Batch 1802144</b>	Diluted at 3 % with water for injection	0 ± 0	practically non irritant

The response obtained for the test item cannot be compared, due to a lack of historical data in that category of product (cosmetic ingredient).

### XIII. STUDY DIRECTOR ATTESTATION

The study **B14 0166** intended to assess the irritant potential of the test item **GPS-S Batch 1802144**, after application to the embryonic hen's egg chorioallantoic membrane (method Het-Cam).

The technique used was an adaptation from the one described by Luepke N.P. and Kemper F.H. (The Het-Cam test: "An alternative to the Draize eye test". Food Chem. Toxicol. 1986, 24, n° 6/7, 495-496).

This method was described in the French order of November 29, 1996, published in the Official Journal of the French Republic of December 26, 1996.

The sponsor asked this study to be carried out according to the principles of the Good Laboratory Practice of in reference to the texts below:

- OECD Principles on Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM (98) 17 and all subsequent consensus documents,
- Directive 2004/10/EC of the European Parliament and of the Council of 11 February 2004 on the harmonization of laws, regulations and administrative provisions relating to the application of the Principles of Good Laboratory Practice and the verification of their applications for tests on chemical substances (OJ No. L50 of 20.2.2004),
- Order of August 10th, 2004 taken to be the application of the article L. 5131-5 of the code of the public health relating to the Good Laboratory Practice for cosmetics product (Official journal n° 218 of September 18th, 2004), Ministry of Health and social welfare system.

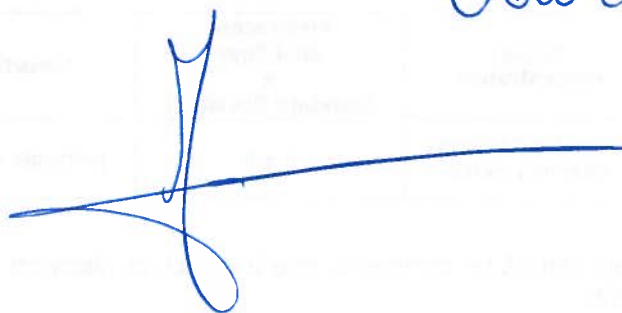
I undersigned, **Sarah JULIENNE**, declare that this study is in accordance with the above-mentioned texts of the Good Laboratory Practice.

Sarah JULIENNE

Date :

Study director :

March 20, 2016



**XIV. QUALITY ASSURANCE ATTESTATION**

I the undersigned, **Florence PROD'HOMME**, for the Quality Assurance Unit, declare that :

- The Quality Assurance Unit ensured the strict application of the Test Facility's current procedures

Audits about the Test Facility were performed once a year. These audits allowed checking whether the Test Facility met the requirements of the authorities or not.

Study-based audits (documentation and process appropriate to this type of study) were carried out; the result of Quality Assurance evaluations was documented and reported to the Study Director and Management as follows:

Dates of audits	Audited phases	Dates of audit report transmission to study Director	Dates of audit report transmission to Management
February 17, 2014	Test conduct*	February 24, 2014	February 24, 2014
March 20, 2014	Final report	March 20, 2014	March 21, 2014

\* Audit conduct on a study of the same type.

The final report fully and accurately reflects the procedures and the data generated during the study.

Only the paper version of the final report is legally acceptable.

Florence PROD'HOMME

Date : 20-03-14

Quality Assurance :

