International Journal of Pharmaceutical Science Invention
ISSN (Online): 2319-6718, ISSN (Print): 2319- 670X
www.ijpsi.org Volume 13 Issue 4 ǁ Jul. –Aug, 2024 ǁ PP. 09-16

Evaluation of the moisturizing effect of a O/W microemulsion (WPE®):Neomist®, a model of human skin maintained in survival mode

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Abstract
We carried out a study using an oil-in-water fluid emulsion based on the WPE® process, which enables up to 40% oil to be combined with water, while retaining an aqueous texture and allowing the active ingredients to be absorbed by the skin.
The work carried out consisted of a study on the skin of healthy volunteers maintained in survival conditions. The results of this study demonstrate the moisturizing effect of WPE® (Neomist®) complexe, with significant results on the skin's glycosaminoglycan and filaggrin content. In our experimental model of altered dehydration on human skin maintained in survival conditions, we were able to assess the positive effects of Neomist® Hydratant.
These results confirm the action of the WPE® process on the efficacy of lipophilic active ingredients into the deep layers of the epidermis and dermis, and thus constitute an original approach to what can be described as "nutricosmetics". Neomist® can thus be considered one of the first nutricosmetics to be presented in aqueous mist form, with a lipid content of almost 40%.
Key words: *human skin model, water plant emulsion, neomist, moisturizing effect,nutricosmétics*

Mots clés : modèle de peau humaine, émulsion végétale à base d'eau, neomist, effet hydratant,nutricosmétique.

Résumé
Nous avons réalisé une étude avec une émulsion fluide huile dans eau basée sur le procédé WPE® qui permet d'associer jusqu'à 40 % d'huile à l'eau, tout en conservant une texture aqueuse et en permettant l'absorption des actifs par la peau.
Les travaux ont consisté en une étude sur la peau de volontaires sains maintenus en conditions de survie. Les résultats de cette étude démontrent l'effet hydratant du complexe WPE® (Neomist®), avec des résultats significatifs sur la teneur en glycosaminoglycanes et en filaggrine de la peau. Dans notre modèle expérimental de déshydratation altérée sur la peau humaine maintenue dans des conditions de survie, nous avons pu évaluer les effets positifs de Neomist® Hydratant.
Ces résultats confirment l'efficacité du procédé WPE® pour la pénétration des actifs lipophiles dans les couches profondes de l'épiderme et du derme, et constituent ainsi une approche originale de ce que l'on peut qualifier de "nutricosmétique".
Neomist® peut ainsi être considéré comme l'un des premiers nutricosmétiques à se présenter sous forme de brouillard aqueux, avec une teneur en lipides de près de 40%.

Date of Submission: 26-06-2024 Date of acceptance: 05-07-2024

I. Introduction

The moisturizing effect of cosmetics is often described in the literature, mainly thanks to their lipid composition [1]. But penetration into the deeper layers of the epidermis and dermis is rarely demonstrated, as the composition of these products is not efficient for that [2].

The notion of cutaneous hydration is confusing because most of the water contained in the skin is found in the dermis but also depends on the state of the *stratum corneum* (most superficial part) of the epidermis.

Water from the bloodstream binds to the proteoglycans of the dermis, the skin's true water reservoir. In fact, due to their high polyanionic charge content, glycosaminoglycans have a high water retention capacity. This water bound by hydrogen bonds (weak bonds) can be mobilized and migrates from the dermis to the epidermis by osmotic phenomenon through epidermal junction, then by diffusion during epidermal

keratinization. A large proportion of this water evaporates in the outermost layers, this is known as insensible water loss or IWL, estimated at 300-400ml/day. Insensible water loss is increased in the event of impaired barrier function.

The hydrophobicity of *stratum corneum* is due to a mixture of hygroscopic substances contained in horny cells called "Natural Moisturizing Factors" (NMF). This NMF is made up of inorganic ions, organic acids, urea and amino acids derived in part from the degradation of filaggrin. In dry skin, the synthesis of profilaggrin slows down, precursor to Filaggrin, whose degradation in the superficial layers of the stratum corneum ensures the supply of NMF. Finally, lipids in the *stratum corneum* lipids, which in particular for ceramides have a real affinity for water due to their hydrophilic sites, and will tend to capture will tend to capture water molecules. Hydration therefore also depends on the water retained in the *stratum corneum* via lipids. Any alteration to the structure of the *stratum corneum* and lipids can lead towater loss and thus to dehydration.

To improve skin hydration, we need to act on several levels:

- Create an impermeable surface film to suppress evaporation,
- Bind water to corneocytes using hygroscopic substances
- Regulate water flow by acting on intercellular lipids
- Increase the number of hyaluronic acid receptors in the epidermis (CD44)
- Increase the quantity of glycosaminoglycans in the dermis

The aim of this study is to demonstrate the efficacy of an oil-in-water mixture of a cosmetic mist containing up to 40% lipophilic substances. The process used to produce this mist is called a Water-Plant-Emulsion (WPE®) and the commercial product is Neomist® hydratant.
The studies carried out will demonstrate the superficial and deep moisturizing properties of these processes/products.

II. Material and methods

1) Composition of lipophilic actives of Neomist® Hydratant

Argan oil (14%), Shea olein (14%) Wheat ceramides (Lipowheat® - 1%)

2) Model of skin maintained in survival and development of an experimental model of TEWL (transepidermal water loss)

Fragments were obtained from plastic surgery (8 different donors, breast or abdominal breast or abdominal plastic surgery, aged between 35 and 55 years). They were deposited in inserts, which were then placed on culture wells. From culture medium specifically adapted to survival maintenance (antibiotics, SVF) was added to the bottom of the wells, with slow diffusion between the two between the two compartments via a porous membrane (3 µm).
An experimental was carried out using 2 sessions (at D0 and D1) of UV A of UV A (6 J/cm^2)and UV B (2 J/cm^2) irradiation, followed by application of LSS à 5%.
From D0 to D5, Neomist® Hydratant (lot 68-D1) was applied topically to the skin surface skinfragments, once a day.
The set was maintained in organ culture for 3 days in a humid oven at 37°C in the presence of5% CO_2.

The cultures were stopped at D5 for Filaggrin immunohistochemical analysis, Hyaluronic AcidReceptor (CD44) and histological analysis of GAGs by Hale staining.

3) Analyses

Three analyses were carried out:

- Immunohistochemical evaluation of the amount of Filaggrin present in the stratum corneum, an interesting approach for assessing the skin's protective capacity against external dehydrating agents.
- Expression of the CD44 receptor for hyaluronic acid, a major skin glycosaminoglycan, in the epidermis.
- The quantity of glycosaminoglycans in the epidermis and dermis after Hale staining.

a) Immunohistochemical analysis of filaggrin

Detection of filaggrin (BT-576 antibody, Clinisciences) was performed using a 2-layerimmunoperoxidase technique (ABC kit, Vector Laboratories) and revealed in AEC.
The intensity of immunohistochemical staining in the granular layer was assessed using a semi-quantitative

histological score:
- score 0: negative staining
- score 1: low intensity of staining
- score 2: moderate intensity of staining
- score 3: high intensity of marking
- score 4: very high intensity of marking
-

b) Evaluation of glycosaminoglycans

Glycosaminoglycans were detected by Hale staining.
A semi-quantitative evaluation using scores was used to highlight any changes in the quantity of glycosaminoglycans in the epidermis and dermis (scores 0 to 4).

c) Evaluation of the hyaluronic acid receptor, CD44

An 80-95 kD transmembrane glycoprotein, CD44, the receptor for hyaluronic acid in the epidermis, can be detected.
Immunodetection was performed using an indirect 3-layer immunoperoxidase technique (ABC Peroxidase kit, Vector Laboratories) and revealed in AEC.
Semi-quantitative scores were used to specify immunostaining topography and intensity.

d) Statistics
A mean was calculated on n = 8 donors. Statistical analysis was performed using the Student's t-test or paired samples test, with an alpha 5% risk.

III. Results

1) Immunohistochemical analysis of filaggrin
The results of filaggrin are shown in Table I (averaged over the 8 donors) and illustrated in Figures 1 to 3.
Treatment for 5 days with Neomist® Hydratant resulted in a significant increase in filaggrin filaggrin expression with a score of 2.68 versus 1.96 for skin whose TEWL had been altered by UV and LSS (p=0.005).

2) Histological identification of glycosaminnoglycans
The results of GAGs are shown in Table II (average obtained from 8 donors) and illustrated in Figures 4 to 6.
In our experimental model with dehydration, we observe a decrease in the amount of glycosaminoglycans of glycosaminoglycans in UV- and LSS-treated skin, in the deep dermis, with a score of 2.2, compared to untreated control skin untreated control skin (score of 2.65; p
= 0.01).
After treatment with Neomist® Hydratant, the quantity of glycosaminoglycans was significantly increased in the epidermis (score of 1.3 versus 0.3 in UV + LSS control skin; p = 0.006) and deep dermis (score of 3.2 *versus* 2.2 in UV + LSS control skin; p = 0.01).

3) Immunohistochemical identification of the hyaluronic acid receptor
CD44 results are shown in Table III (average obtained on the 8 donors) and illustrated inFigures 7 to 9.
In our experimental model, the quantity of hyaluronic acid receptors is significantly reduced: a score of 2.8 *versus* 3.87 in control skin (p =0.03).
After treatment with Neomist® Hydratant, CD44 receptor expression tends to increase, but notsignificantly.

IV. Conclusion

In our experimental model of altered TEW with dehydration on surviving human skin, we were able to assess the positive effects of neomist® Hydratant. Indeed, we showed an increase in the quantity of glycosaminoglycans in the epidermis and dermis by Hale staining, testifying to improved skin hydration. We also observed an increase in Filaggrin, ensuring correct EIP. In this way, we have indirectly demonstrated that Neomist® Hydratant restores the cutaneous barrier.

The bioavailability of the lipid actives is granted by the WPE® formulation, consisting of a O/W microemulsion achieved with a natural surfactant (saponins of vegetal origin) and a highly efficient mixing process generating micro drops (ultradrops®) [4].

We have demonstrated the interest of a synergy between high concentrations of natural lipids (argan oil, shea olein and wheat ceramides, total 29% in the formula) and an efficient vectorization system (WPE®) for

creating a new generation of effective and convenient cosmetic products.

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Table I: Immunohistochemical evaluation of filaggrin (intensity of labelling in the granular granularlayer)

	Intensity
Skin Control	**1.8** ± 0.7
Skin + **UV**+ **LSS** (skin control)	**1.96** ± 0.6
Skin + **UV+ LSS + Neomist®**	**2.68** ± 0.5 * p = 0.005

* statistically significant difference from UV control skin (paired Student's t test, $p < 0.05$)

Table II: Histological assessment of glycosaminoglycan levels after Hale staining (intensity ofepidermal and dermal staining using semi-quantitative scores).

	Epidermis	**Superficial dermis**	**Middle-deep dermis**
Skin Control	**0.65** ± 0.6	**2.87** ± 1	**2.65** ± 0.48
Skin + UV+ **LSS** (skincontrol)	**0.3** ± 0.4	**2.6** ± 0.78	**2.2** ± 0.7 *** p = 0.01**
Skin + **UV+ LSS + Neomist®**	**1.3** ± 0.9 * p = 0.006	**3.1** ± 0.5	**3.2**± 0.47 * p = 0.01

*statistically significant difference from control skin (paired Student's t test, $p < 0.05$)
* statistically significant difference from UV-treated control skin (paired Student's t test, $p < 0.05$)

Table III: Immunohistochemical evaluation of CD 44 expression (intensity and topography of epidermal labeling using semi-quantitative scores)

	Topography	**Intensity**
Skin Control	**3.87** ± 0.3	**2.6** ± 0.5
Skin + UV + **LSS** (skin control)	**2.8** ± 1.3 *** p = 0.03**	**2.45** ± 0.4
Skin + **UV + LSS + Neomist®**	**3** ± 1.2	**2.78** ± 0.4

*: statistically significant difference from control skin (paired Student's t test, $p < 0.05$)

Figure 1: Filaggrin immunohistochemistry (x400): Expression of filaggrin in the granular layer of theskin Skin control

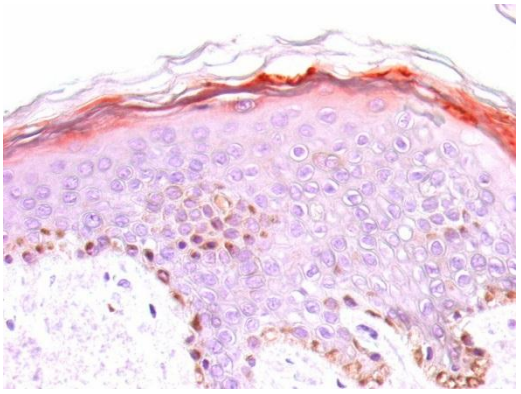

Figure 2: Filaggrin immunohistochemistry (x400): Expression of filaggrin in the granular layer of theskin Control skin (experimental skin dehydration by UV + LSS).

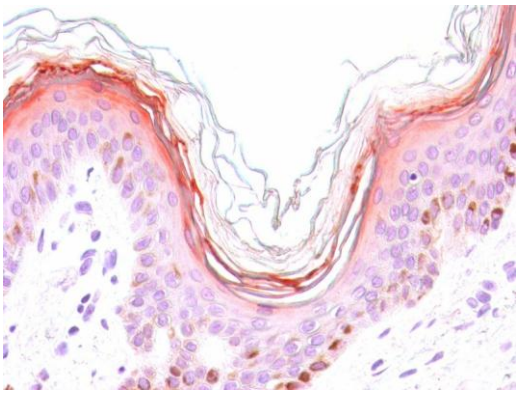

Figure 3 Filaggrin immunohistochemistry (x400): Expression of filaggrin in the granular layer of theskin Peau + UV + LSS + Neomist®

No change in Filaggrin expression compared with control skin

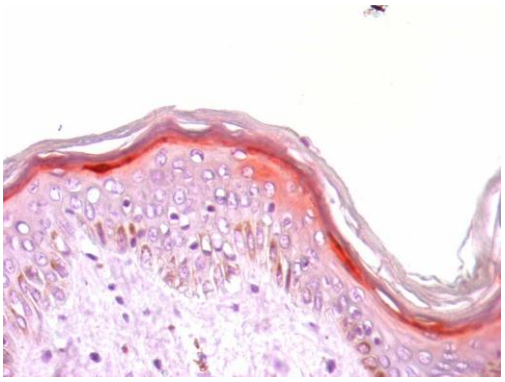

Figure 4: Histological demonstration of glycosaminoglycans by Hale staining (x400)
Skin control

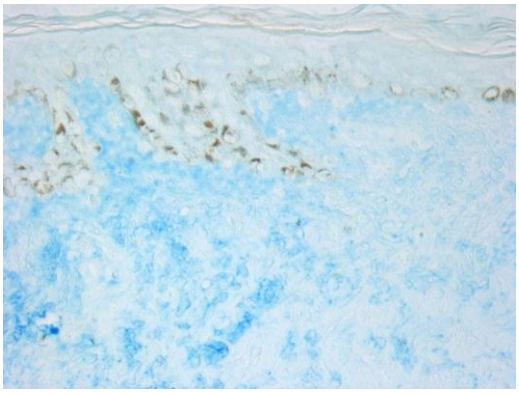

Figure 5: Histological demonstration of glycosaminoglycans by Hale staining (x400)

Skin experimental ageing
Glycosaminoglycans decrease

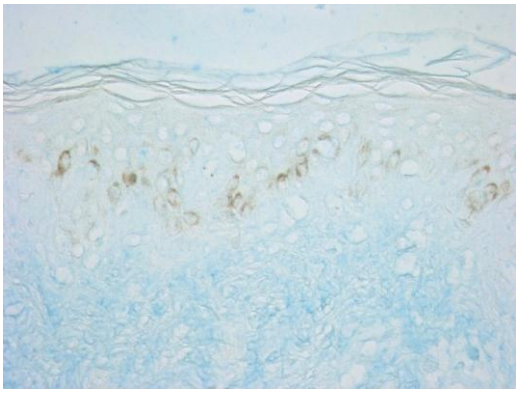

Figure 6: Histological demonstration of glycosaminoglycans by Hale staining (x400)

Skin + UV + LSS + Neomist®
Glycosaminoglycans increase

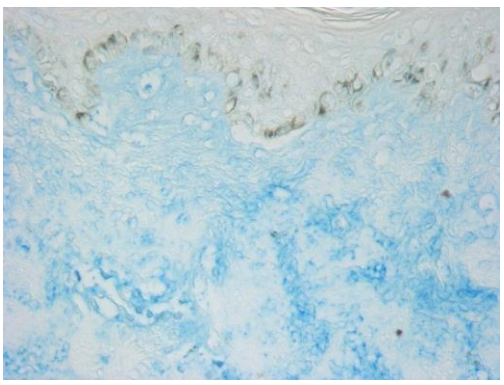

Figure 7: CD44 immunohistochemistry (x400))

Skin control
High CD44 expression in keratinocyte cell membranes throughout the epithelium

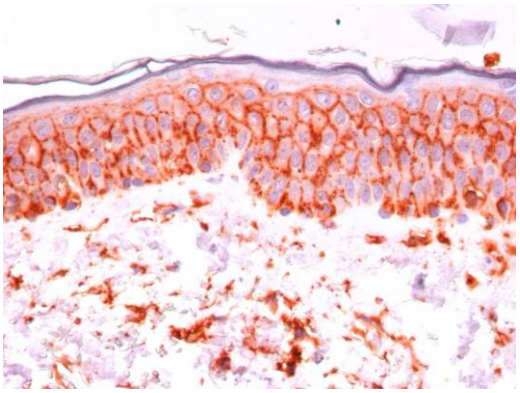

Figure 8: CD44 immunohistochemistry (x400))

Skin experimental ageing Decreased CD44 expression in the epithelium

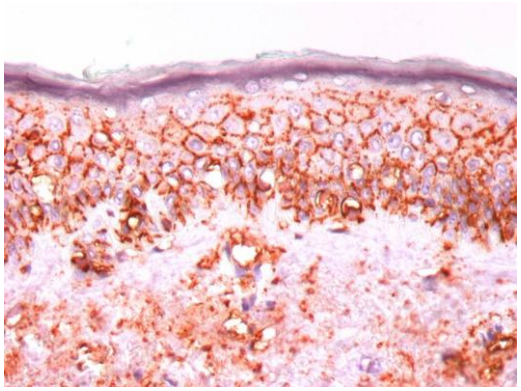

Figure 8: CD44 immunohistochemistry (x400))

Skin + UV + LSS + Neomist®