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[TRANSLATION]

Menthyl nicotinate

High rate of skin absorption and time-release delivery of Vitamin B3 (Niacin)

Gabriele Segalla[1], Silvana Giardina[2], Gioia Bizzaro[2]
[1] Multichem R&D - segalla@multichem.it
[2] Complife Group

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Abstract

Menthyl nicotinate is a new multifunctional, sensory active ingredient that activates the microcirculation of the skin. It has been proven to be effective as an antioxidant, antipollutant, detoxifying and protective agent. This *in vitro* study on a reconstructed human epidermis (RHE) model investigates menthyl nicotinate's fast penetration kinetics through the skin, and how its subsequent hydrolysis releases menthol and niacin (vitamin B3) within 24 hours of its application. The total release of niacin was analyzed at different time points, and the release curve was plotted. Niacin slow diffusion in the underlying skin layers, where it interacts with the epidermal and dermal biological structures, was also studied. Such time-dependent release of niacin and menthol, in an equimolar ratio, prevents the niacin-flush effect that is usually observed with other nicotinate-based formulations. It also allows menthyl nicotinate to gradually interact with skin thermoreceptors, alone or in combination with other sensory agents, to provide a characteristic pleasurable and modulated effect.

Introduction

This study was designed to investigate menthyl nicotinate's ability to penetrate the skin barrier as an active cosmetic ingredient (commercial brand name: NICOMENTHYL®; INCI name: Menthyl Nicotinate), in a reconstructed human epidermis (RHE) model.
Based on previous *in vivo* studies of formulations containing this substance, our hypothesis was that menthyl nicotinate, after being applied on the skin, rapidly penetrates through

the stratum corneum and is rapidly hydrolyzed into niacin and menthol. These compounds then slowly diffuse to the lower layers of the skin, where they can interact with both epidermal and dermal structures.

To investigate this hypothesis, the present *in vitro* study assessed the absorption of a 3% menthyl nicotinate caprylic/capric triglyceride solution and characterized the skin penetration kinetics of this ingredient. Moreover, the study also analyzed the distribution kinetics of the constituents in the skin by dosing the amount of unbound niacin and carrying out appropriate stoichiometric calculations.

Niacin, or nicotinic acid, was first described by the Austrian chemist Ugo Weidel in 1873 while studying nicotine. Weidel managed to synthetize it by oxidizing nicotine with nitric acid. This was the only reason this substance was then called "nicotinic" acid, even without being biochemically or toxicologically related to the well-known tobacco alkaloid.

The discovery that niacin deficiency was the cause of pellagra in humans and of black tongue in dogs was made many years later, in 1937, by the American chemist Conrad Arnold Elvehjem, who isolated it from a deproteinized liver extract. From that moment on, niacin was regarded as the third vitamin in the B group and was therefore called "Vitamin B3" or "Vitamin PP", an acronym for *"Pellagra Preventing"*.

Some years later, in 1942, the American Medical Association gave this substance a more popular name that was less misleading than nicotinic acid, to prevent illogical, unfounded associations between the harmful effects of nicotine and the therapeutic ones of vitamins: NIACIN, from the first two letters of NIcotinic ACid, and the last two letters of vitamIN.

Niacin plays an essential metabolic role throughout the animal kingdom. It reduces the synthesis of triglycerides and cholesterol, prevents cardiovascular diseases, helps maintain skin integrity and nervous system function, and is used to treat schizophrenia and depression.

In the skin, once niacin has crossed the cell membrane of the keratinocytes, it triggers a complex cascade of biochemical reactions that produce one of the most important cellular coenzymes, NAD^+ (nicotinamide adenine dinucleotide). NAD^+ plays a pivotal role in hundreds of enzymatic reactions, including those related to energy (ATP) production, many reduction-oxidation metabolic reactions, the Krebs cycle, as well as many others involved in cell signaling within the immune system, DNA repair, epithelial cell renewal, the synthesis of particular lipids (ceramides) essential to skin barrier functionality.

Within this complex frame of metabolic actions and interactions, perhaps one of the more important properties of niacin is its "detox" action. Its capability of removing xenobiotic substances from the skin was first described by L.R. Hubbard in 1977 (**1**,**2**). Since its discovery, the Hubbard method, based on using niacin and other vitamins in increasing doses, was extensively studied and applied in many cases of intoxication caused by even prolonged exposure to highly toxic and carcinogenic agents (**3**). As an example, niacin was used with considerable success to detoxify emergency team members who removed the debris from the contaminated area at World Trade Center after the 9/11 attack. These workers were exposed, to a variable extent, to highly toxic chemicals including polychlorinated biphenyls (PCBs), polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzodioxins (PCDDs) (**4**).

Niacin not only acts as a protective agent against chemical agents, but also against UV radiation. Recent studies, in fact, have shown that the niacin-dependent NAD^+ increase in keratinocytes has a preventive and reparative action on skin damage caused by excessive solar radiation exposure (**5**,**6**).

Menthol, the other constituent that is released following menthyl nicotinate hydrolysis, shows a substantial balsamic and lenitive action. As menthol is released in the skin in an equimolar ratio to niacin, it significantly soothes or even eliminates the niacin flush effect, without inhibiting its vitaminic and biochemical properties. In contrast to the effects of other nicotinates like methyl- or ethyl-nicotinate, the menthyl ester, at the recommended doses, does not create significant hyperemia or skin irritation, and does not lead to sensitization, as demonstrated by several *in vitro* and *in vivo* safety tests carried out using either the pure ingredient or formulations with a concentration up to 3-5%.

This study shows how menthyl nicotinate, because of its rapid transcutaneous penetration (about 90% within 24 hours) and of its concomitant slow hydrolysis, may be a novel and noteworthy topical, time-release vitamin B3 carrier providing significant protective, antioxidant, anti-free radical, detoxification, and antipollution actions (**7**). This time-modulated effect, according to the results of the present study, lasts over a whole day, preserving and protecting the integrity and functionality of the skin barrier for many hours after the application and reducing transepidermal water loss (TEWL). This may greatly benefit people with sensitive skin, preventing damage from exposure to excessive solar radiation or harmful chemicals, particularly in urban and industrialized areas.

Mechanism of action

Menthyl nicotinate is an ester, i.e. the product of the reaction between an alcohol (menthol) and a carboxylic acid (nicotinic acid). As a consequence, when it crosses the skin barrier, it is hydrolyzed by the enzyme esterase that splits it into its original constituents, menthol and niacin (***Fig.1***)

One of the more interesting consequences of the slow hydrolysis of menthyl nicotinate is the action it exerts on cutaneous nerve endings, producing a unique, delicate, and prolonged sensory effect. Within few minutes from application, indeed, the slow gradual release of menthol produces a pleasurable sensation of cold, resulting from the activation of two ionic channels, TRPM8 and TRPA1, which act as cold-sensitive thermoreceptors (**8**). The niacin component, beyond providing a beneficial amount of vitamin B3 to the skin, activates the warmth-sensitive TRPV1 and TRPV3 thermoreceptor channels, already at body temperature (**9**). The result of such concomitant, synergic activation of cold receptors by menthol and warmth receptors by niacin is perceived as a peculiar combination of alternating cold and warm sensations, that varies according to the menthyl nicotinate concentration, and is described by users with several different adjectives, such as: intense, deep, long-lasting, fresh, and tingling.

Materials and methods

This study was carried out on reconstructed, differentiated human epidermis (EpiDerm™), that was maintained in a culture medium provided by its manufacturer. The tissue is a model of the epidermis, with an area of 0.6 cm^2, reconstructed from human keratinocytes. Cells are grown at the air-liquid interface on a polycarbonate filter, in a medium having a well-defined chemical composition. In such conditions, the reconstructed tissue, being metabolically active, reproduces the in vivo anatomical structure of human epidermis, with all its functional properties. These features make it a valid alternative to other matrices used for penetration studies.

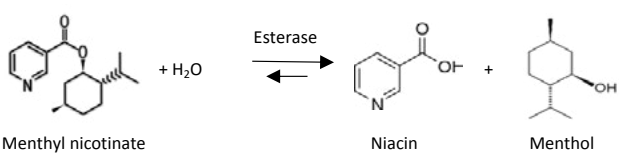

Figure 1 – Hydrolysis of menthyl nicotinate.

Additionally, it is a standardized model that guarantees realistic and replicable results, with no ethical implications (**10**,**11**,**12**,**13**). The manufacturer performed appropriate quality controls on the tissue samples provided, showing that they reveal a normal histological appearance (i.e. no significant alterations), regular cell viability, functional skin barrier integrity, absence of microorganisms or infectious agents. The tissues were maintained throughout the experiments in 12 well plates containing 1 ml of medium, under standard cell culture conditions (37°C, 95% RH, 5% CO_2). The product was tested as a 3% menthyl nicotinate caprylic/capric triglyceride solution. A volume of 100 µL of this solution (corresponding to 3000 µg of menthyl nicotinate) was applied to the reconstructed epidermis for up to 24 hours. The quantity of menthyl nicotinate (Nicomenthyl * - hereafter abbreviated to NM) and niacin in the tissue (measured after tissue homogenization with a lysis buffer) and in the underlying medium was measured after 15 and 30 minutes and after 1, 2, 4, 8, and 24 hours of application using high-performance liquid chromatography (HPLC). As the amount of product being applied at baseline was known, HPLC measurements allowed calculation of the percentage of menthyl nicotinate applied to the tissue surface that penetrated the reconstructed epidermis at each time point.

Results and discussion

Menthyl nicotinate showed, in the experimental model used in the present study, high penetrating power over the monitored time period (24 hours). Indeed, the amount of the compound that penetrated the reconstructed epidermis by the end of the experiment was about 90% of the dose initially applied on the tissue surface (***Table 1*** and ***Figure 2***). The absorption level already reached about 50% after 30 minutes from the application. No undissociated menthyl nicotinate was detected in the tissue medium underlying the epidermis, showing that the ester was confined in the superficial skin layer throughout the test period.

As the NM level increased in the epidermis, a progressive rise in the quantity of niacin was observed both in the epidermis and in the underlying medium. Niacin, in contrast to menthyl nicotinate, can diffuse into the deeper skin layers. The amount of unbound niacin that was gradually released over the 24 hours following the application of menthyl nicotinate was equal to 254 µg, that is to say 18% of the total niacin (1413 µg) initially present in the ester (***Table 2*** and ***Figure 3***).

The amount of niacin increased over time both in the epidermis and the underlying medium. During the test, the ratio between the content of niacin within the two compartments changed over time (***Figure 3***). At the first time points, most niacin was in the superficial zone. Over time, the relative quantity of niacin in the medium underlying the epidermis increased, indicating a gradual, progressive diffusion of unbound niacin, capable of reaching the tissues underlying the epidermis. We then calculated the amount of menthyl nicotinate that penetrated after its application to the epidermis, as well as the amounts of niacin and menthol that were released by hydrolysis. To do this, we performed projections and stoichiometric calculations that considered the molar amounts of menthol and niacin contained in menthyl nicotinate, as well as the sum of undissociated menthyl nicotinate found in the epidermis and the total unbound niacin released in the experimental model (***Table 3*** and ***Figure 4***).

Conclusions

The results of this preliminary *in vitro* study show that NM displays rapid penetration kinetics. Indeed, penetration is essentially complete within 24 hours after the application. The concomitant slow hydrolysis of the ester with unbound niacin formation increases progressively over time: it begins within 15 minutes of the application and continues over the first hour almost exclusively within the superficial epidermal layers. After that, the released niacin diffuses into the underlying layers where it becomes available to exert its well-known effects, which have been extensively described in literature. The quantity of this unbound niacin is about the 18% of the total initial niacin contained in the undissociated ester. This result is undoubtedly noteworthy if one compares it, for example, to the transcutaneous absorption of nicotinamide (also known as niacinamide, another form of vitamin B3) that, over 24 hours, represents only 2.2% of the initially applied dose (**14**). This study therefore highlights two mechanisms: the fast penetration of menthyl nicotinate into the epidermis, followed by its slow esterase-mediated hydrolysis and the consequent, progressively increasing niacin release over time even beyond the epidermal compartment. This peculiar mechanism of action is certainly of interest for future biochemical and cosmetic studies. Yet, it already can be applied in practice to exploit the functional cosmetic properties of this ingredient, including its sensory effects and carrier abilities, which can be exploited either alone or in combination with other cosmetic actives.

Transcutaneous absorption - NM 3000 µg			
Time point	NM (homogenate) µg	NM (medium) µg	NM% total epidermal penetration
15 min	605.0	-	20.2%
30 min	1615.2	-	53.8%
1 h	1993.2	-	66.4%
2 h	2346.7	-	78.2%
4 h	2481.7	-	82.7%
8 h	2557.7	-	85.3%
24 h	2633.2	-	87.8%

Table 1 – Quantity of NM in the different experimental compartments (homogenates and tissue medium), and the corresponding penetration (%) in the epidermis.

Niacin release following hydrolysis of NM 3000 µg			
Time point	Niacin (homogenate) µg	Niacin (medium) µg	Niacin (total) µg
15 min	19.2	1.3	20.5
30 min	43.7	4.8	48.5
1 h	55.8	10.3	66.1
2 h	89.1	16.7	105.9
4 h	122.8	26.4	149.2
8 h	150.7	41.2	191.9
24 h	186.5	67.6	254.1

Table 2 – Quantity of Niacin in the different experimental compartments (homogenates and tissue medium).

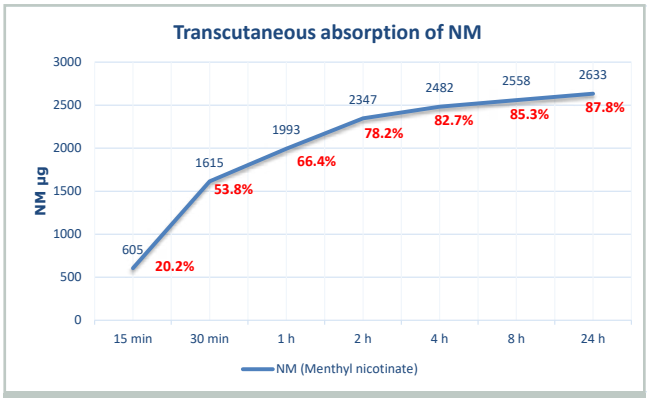

Figure 2 – Kinetic of NM penetration in the in vitro reconstructed epidermis system.

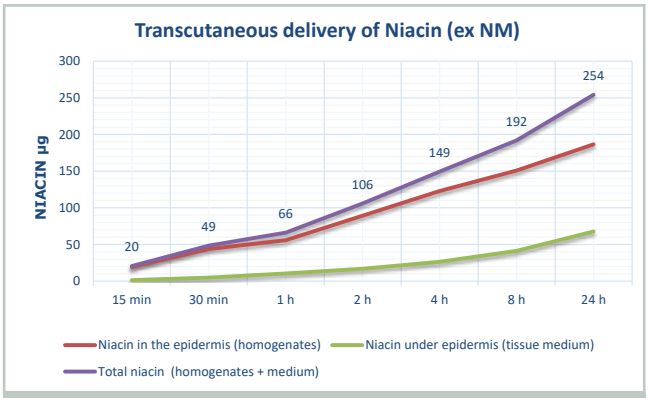

Figure 3 – Kinetic of Niacin diffusion in the in vitro reconstructed epidermis system.

NM – Absorption and hydrolisis				
Time point	NM being absorbed µg	Non-hydrolized NM µg	Total unbound Niacin µg	Total unbound Menthol µg
15 min	605.0	2956.5	20.5	26.0
30 min	1615.2	2897.0	48.5	61.6
1 h	1993.2	2859.8	66.1	83.8
2 h	2346.7	2775.2	105.9	134.4
4 h	2481.7	2683.3	149.2	189.3
8 h	2557.7	2592.5	191.9	243.6
24 h	2633.2	2460.5	254.1	322.6

Table 3 – Quantity of NM being absorbed, of non-hydrolyzed NM, and of niacin and menthol produced by hydrolysis.

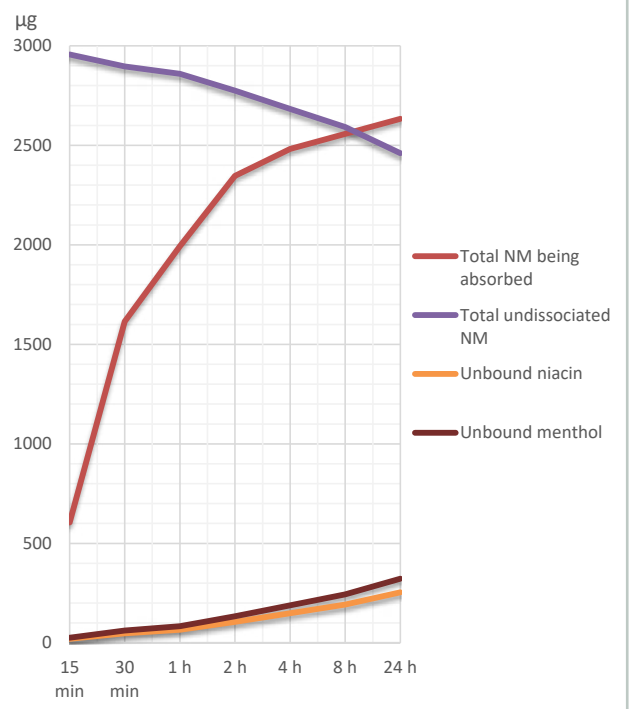

Figure 4 – Kinetic of NM penetration and of its concomitant hydrolysis into niacin and menthol.

Specifically, menthyl nicotinate may be regarded as a kind of biochemical modulator: a substance that can modulate and prolong the sensory effects of warming agents (like vanillyl butyl ether, capsaicin, etc.), cooling agents (like menthyl lactate, isopulegol, etc.), and vasodilators (such as L-arginine, methyl nicotinate, ethyl nicotinate, etc.), smoothening their aggressiveness and irritating effects. Further studies should be carried out to investigate the kinetics of menthyl nicotinate hydrolysis up to completion, and to assess how niacin distributes over time within the different cutaneous anatomic compartments, extending the experimental time beyond the 24 hours of the present study. This will allow us to achieve a more fine and detailed characterization of the interesting, and somewhat surprising, functional properties of this novel cosmetic active ingredient.

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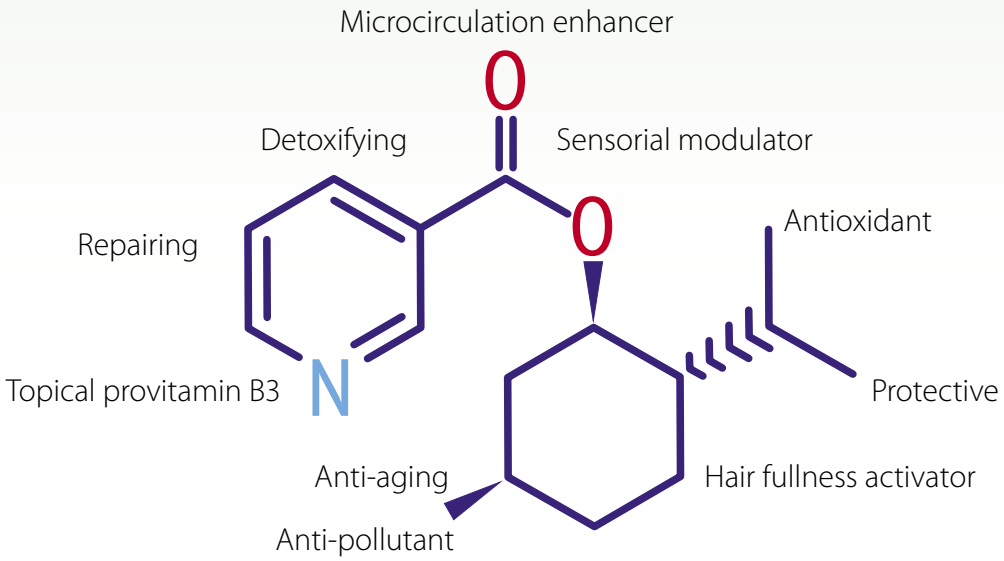

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MULTICHEM R&D SRL
Via G. Marconi 37 - 20089 Rozzano (MI) - Italy
www.multichemrd.com
info@multichem.it