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Effects of ultraviolet radiation on the kinetics of *in vitro* percutaneous absorption of *lavender oil*

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ABSTRACT

The purpose of the present study is to investigate the influence of ultraviolet radiation on the rat skin absorption of *lavender* essential oil. The pure oil was extracted from *Lavandula angustifolia* by steam distillation. The chemical composition of lavender oil showed that terpenes are major compounds. *In vitro*, the essential oil was applied onto the rat skin. The amount of the compounds was determined using gas chromatography. Similarly, the amount of these compounds was analyzed for the skin exposed to ultraviolet radiation (UVAI) after 4, 8, 12 and 24 h. Our study demonstrated that the penetration profiles showed a cycle of charge–discharge (4 h/4 h, respectively). Our data point to the presence of reversible change in stratum corneum behavior. Interestingly, the ultraviolet radiation altered the cycle (charge–discharge) for terpenes (low lipophilicity) and increased the charge time. However, for terpenes (high lipophilicity), the ultraviolet radiation decreased the charge amplitude.

1. Introduction

The absorption of auxiliary ingredients from pharmaceutical formulations is one of the least explored processes (Cal et al., 2001; Cal and Sznitowska, 2003). This is also true for percutaneous penetration enhancers, a special kind of excipients which penetrate into the skin and the promote diffusion of drugs as a consequence of the changes in the structure of stratum corneum, a barrier layer of the skin (Cal and Sznitowska, 2003). Terpenes are absorption promoters frequently used in numerous studies, but the kinetics of their transdermal absorption has not been well-documented yet, in spite of the fact that terpenes are used in cosmetic and dermatological products not only for their promoting effect but also as fragrances or active substances (Williams and Barry, 1991). They are considered as nontoxic; however, they are well-known sensitizers and, due to a very broad biological activity, other side effects cannot be excluded (Schempp et al., 2002). Therefore, their penetration through the skin should be controlled.

The mechanism of the changes in the stratum corneum (SC) is a subject of most publications on terpenes and skin (Williams and Barry, 1991; Buck, 2004). Alone, or in mixtures, terpenes promote the percutaneous penetration of lipophilic as well as hydrophilic drugs. The range of Log *P* values for terpenes is wide, from 1 up to 6, and the linear relationship between Log *P* and the enhancement effect towards penetration of model drugs was reported (El-Kattan et al., 2001). There are only few published studies on absorption of terpenes into the skin. In addition, the skin is constantly exposed to pro-oxidant environmental stresses which can influence the percutaneous absorption of many compounds such as terpenes. Indeed, the skin is constantly exposed to the ultraviolet radiation including exposure to the sun, the use of sun-tanning devices and during a phototherapy meeting (Tzaneva et al., 2001; Herrling et al., 2006; Mantena and Katiyar, 2006). Terpenes are major constituents of essential oils such as lavender oil obtained from *Lavandula angustifolia* (Cal et al., 2001; Cavanagh and Wilkinson, 2002). It is used for its sedative and spasmolytic effects (Kim and Lee, 2002).

The purpose of the present study was to determine the *in vitro* percutaneous absorption kinetic of main components of lavender oil and the influence of ultraviolet radiation on its kinetics.

2. Materials and methods

2.1. Essential oil extraction

Dried plants of *L. angustifolia* (3 kg) were coarsely cut and extracted by ultrasound assisted steam distillation. The extraction duration was 3 h. The yield was 3.40% optimized for a relative pressure of 0.4 bar. The essential oil obtained is a pale-yellow liquid with a slightly camphoraceous odor.

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2.2. Skin penetration studies

In vitro skin permeation experiments were carried out using cell diffusion. The dorsal skin of Wistar rat (180–200 g) was shaved by an electric clipper a night before of experiment and then the excised skin was immediately mounted on the receptor compartment with the SC-side facing upwards into the donor compartment and the dermal side facing downwards into the receptor compartment. The receptor compartment was filled with 10 mL of normal saline and stirred constantly using a mini magnetic stirrer. The temperature of the jacketed diffusion cell was regulated by water thermostat maintained at 37 °C.

The diffusion area of the skin was 3.14 cm^2. Following the 30 mn equilibration, the lavender oil (500 mg) was applied onto the skin. The donor compartment was occluded with Parafilm (Sigma–Aldrich, Steinheim, Germany). The essential oil was left on the skin surface for 4, 8, 12 and 24 h. After that, it was removed, and the skin was rinsed shortly with ethanol. The SC layers were separated using fragments of an adhesive tape. Collected samples of SC as well as the remaining skin epidermis/dermis (ED) were extracted by shaking in 5 mL of ethanol for 24 h at room temperature in tightly closed vials. The ethanol extracts were collected into chromatographic vials and stored at 4 °C before the chromatographic analysis. The acceptor medium was extracted by diethylic ether and collected for analysis. The absorption experiment was repeated in triplicates. In order to study the effect of ultraviolet radiation on percutaneous absorption, we adopt the same protocol except that the system was exposed to an ultraviolet lamp with a wavelength of 366 nm which characterize the UVAI band.

2.3. The qualitative and quantitative analysis of lavender oil and extracts

Lavender oil was analysed by gas chromatography using a Hewlett-Packard (HP) 6890 with the flame ionisation detector and split–splitless injector. The chromatographic conditions were as follows: the detector temperature was 300 °C; the initial oven temperature was isotherm at 35 °C for 10 min, increasing gradually 3 °C/min up to 205 °C and isotherm at 205 °C for 10 min. We used a HP-Innowax capillary column having a polar stationary phase (polyethylene glycol). Chemical components were identified by co-gas chromatography of the essential oils with authentic substances, and by calculating their retention indices.

Table 1
Main components of the essential oils of *L. angustifolia*.

Compounds	%	Compounds	%
α-Pinene	0.68	Linalool	25.18
α-Thujene	0.18	Linalyl acetate	21.71
Camphene	0.81	Terpinene-4-ol	1.60
β-Pinene	0.43	β-Caryophyllene	1.20
Δ-3-Carene	1.43	α-Humulene	1.70
1-8-Cineol	14.12	Germacrene-D	1.10
γ-Terpinene	2.03	α-Terpineol	2.20
E-β-Ocimene	1.25	Borneol	1.14
p-Cimene	1.40	Neryl acetate	0.53
Terpinolene	0.58	Geraniol	0.55
cis-Linalool oxide	1.27	p-Cymene-8-ol	0.32
trans-Linalool oxide	0.82	Carvacrol	2.34
Camphor	11.15		

The percentage composition of the essential oil was computed from gas chromatography peak areas using a HP CHEMSTATION data system. The main components of the lavender oil in the extracts of SC, ED and acceptor medium were identified and their concentration was calculated from their GC peak areas using a HP CHEMSTATION.

2.4. Data analysis

The amounts of terpenes extracted from the skin were expressed per 1 cm^2 area. Statistical analysis was performed using one-way analysis of variance (ANOVA), and the differences were considered significant at $P < 0.05$.

3. Results

3.1. Chemical composition of lavender oil

The chemical identification and the quantitative estimation of lavender oil showed that it contained five main components: linalool (25.18%), linalyl acetate (21.71%), 1,8-cineol (14.12%), camphor (11.15%) and carvacrol (2.34%) (Table 1).

3.2. In vitro percutaneous absorption of lavender oil

The percutaneous absorption of lavender oil was studied by the determination of its main components quantities that was accumulated during the times of exposition, and the system was protected against evaporation. During different times, no terpenes

Table 2
Absorption of terpenes (μg/cm^2) into rat skin layer.

	Time of exposure skin layer[a]			
	4 h	8 h	12 h	24 h
Linalool				
SC	483 ± 141	327 ± 37	110 ± 35.50**	884 ± 462
ED	309 ± 66	534 ± 198	507 ± 9	929 ± 94*
Linalyl acetate SC	427 ± 127	235 ± 76	101 ± 35**	670 ± 319
ED	140.50 ± 7	286 ± 112	236 ± 27	464 ± 19**
Carvacrol				
SC	123 ± ±44	101 ± 13	10 ± 5	65 ± 25
ED	27 ± 6	88.5 ± 44	42 ± 5	83 ± 17*
Camphor SC	184 ± 55	125 ± 17	37 ± 11**	976 ± 629
ED	163 ± 40	244 ± 96.50	251 ± 5	557 ± 107*
1,8-Cineol SC	155 ± 54	133 ± 21	114 ± 73	340 ± 207
ED	120 ± 35	183 ± 88.50	189 ± 24	641 ± 197

Determinate from experiments repeated in triplicates independently (mean ± S.E.M., $n = 3$).
8 h vs 4 h, 12 h vs 4 h and 24 vs 4 h
[a] SC: stratum corneum; ED: epidermis and dermis.
* $p < 0.05$.
** $p < 0,01$.

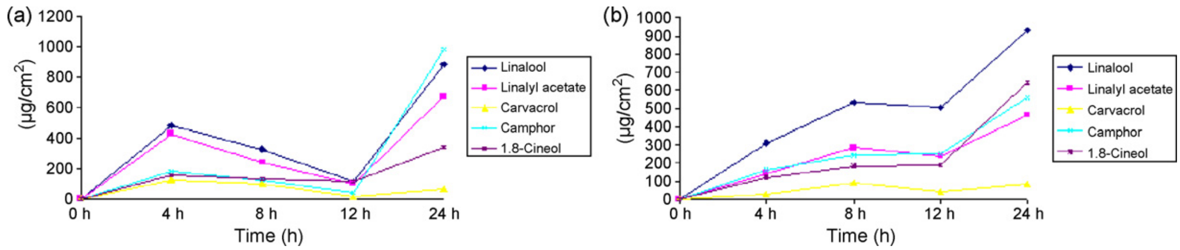

Fig. 1. The amounts of terpenes absorbed to the skin layers in relation to the duration of absorption: (a) in SC, (b) in ED. SC: stratum cornum; ED: epidermis-derma,

were detected in the acceptor fluid. The amounts determined in the separated skin layers after 4, 8, 12 and 24 h (Table 2). The profiles of cumulated terpenes amounts in SC and ED are presented in Fig. 1. The concentrations were not normalized in respect of the collected SC mass.

The terpenes penetrated easily into the skin but with different amounts. However, the amount of all terpenes increased during the first 4 h, then decreased from 4 until 12 h and finally increased again at 24 h. The Fig. 1 shows the percutaneous penetration profile of the main components of lavender oil through the SC (a) and ED (b) in relation to the duration of the absorption.

The analysis of Fig. 1 revealed three sections. First, we observed an increase of the compounds until 4 h of absorption. Second, between 4 and 8 h, the compounds decreased linearly to reach a minimum at 12 h. Finally, from 12 until 24 h the compounds increased again.

3.3. *In vitro percutaneous absorption of lavender oil under ultraviolet radiation*

The percutaneous absorption of lavender oil with ultraviolet radiation (366 nm) was studied by the determination of its main component quantities accumulated during the times of exposition, and the system was protected against evaporation. During different times, no terpenes were detected in the acceptor fluid. The amounts determined in the separated skin layers after 4 and 8 h are presented in Table 3. The concentrations were not normalized in respect of the collected SC mass.

Table 3
Influence of UVAI radiation on terpenes absorptions of (μg/cm^2) into rat skin layer.

	Time of exposure Skin layer[a]	
	4 h	8 h
Linalool		
SC	169 ± 37	218 ± 90[*]
ED	176.50 ± 12[*]	317 ± 41
Linalvl acetate		
SC	147 ± 40	76 ± 4[**]
ED	58 ± 7	75 ± 12
Carvacrol		
SC	20 ± 5	11 ± 2
ED	12 ± 2	14 ± 2
Camphor		
SC	41 ± 13	129 ± 69
ED	102 ± 6	166 ± 10[*]
1,8-Cineol		
SC	12 ± 1	127 ± 83
ED	93 ± 4	166 ± 11.50[*]

Mean ± S.E.M., $n = 3$. 8 h vs 4 h.
[a] SC: stratum corneum; ED: epidermis and dermis.
[*] $p < 0.05$.
[**] $p < 0.01$.

Figs. 2 and 3 allow us to compare the absorption profile of each terpene with or without UVAI radiation from 0 to 4 h and 4 to 8 h in order to detect the influence of these radiations on lavender oil percutaneous absorption in SC and ED.

4. Discussion

The present study was undertaken in order to investigate the influence of ultraviolet radiation on rat skin absorption kinetic of lavender essential oil. Firstly, we have determined the chemical composition of lavender oil. Our results showed that terpenics compounds were main constituents of the essential oil: linalool (25.18%), Linalyl acetate (21.71%), 1,8-cineol (14.12%), camphor (11.15%) and carvacrol (2.34%). The chemical composition analysis of essential oil for the same specie carried out by Evandri et al. revealed the presence of the same constituents but our data were lowed than reported by Evandri et al.; 43.10% for linalyl acetate, 32.70% for linalool (Evandri et al., 2005). This difference in the proportion can be due to the genetic make-up of each cultivar, climatic conditions, storage time, in addition to the distillation and extraction process.

Secondly, we have studied *in vitro* the percutaneous absorption of lavender oil by determination of its main components quantities accumulated during the times of exposition. The amounts of terpenes found in different layers reflected its affinity for biological membranes that may be represented by its lipophilicity. Usually, the lipophilicity of a compound can be quantitatively characterized by Log *P*, the logarithm of its *n*-octanol/water partition coefficient (Balogh et al., 2005). The molecule partitioning between an aqueous phase and a lipid phase conditioned its biological properties and especially its passage through biological membranes (Carpy, 1999). Thus, Log *P* value was the most important parameters for interpreting percutaneous absorption processes of terpenes. According to the literature, Linalyl acetate was the most lipophilic compound ($\text{Log}\,P = 4.12 \pm 0.40$) followed of carvacrol ($\text{Log}\,P = 3.64$).

Linalool was moderately lipophilic ($\text{Log}\,P = 3.28 \pm 0.26$). However, 1,8-cineol ($\text{Log}\,P = 2.82 \pm 0.27$) and Camphor ($\text{Log}\,P = 2.10$) were less lipophilic compounds (Ultee et al., 2002; Rytting et al., 2005; Cal, 2006). Log *P* value reflected well the percutaneous absorption profile; a less important absorption for the compounds with $\text{Log}\,P > 3$ (linalyl acetate and carvacrol). The fastest and progressive penetration into all skin layers was observed for Linalool with its Log *P* about 3. A weak absorption for the compounds with $\text{Log}\,P < 3$ such Camphor and 1,8-cineol which have the same absorption profile. Indeed, according to Cal et al. (2006), the absorption of investigated terpenes into the SC is greater if their Log *P* value was close to 3. In fact, the stratum corneum was an hydrophobic medium but more polar than octanol (Williams and Barry, 1991) this explains the weak diffusion of linalyl acetate although it was the most lipophilic compound ($\text{Log}\,P = 4.12$). The better penetration for Linalool in stratum corneum was due to its Log *P* value ($\text{Log}\,P = 3.28$), its good solubility in water (1.33 mg mL^{-1}) (Cal et al., 2006) and its amphiphilic structure. In addition, the pres-

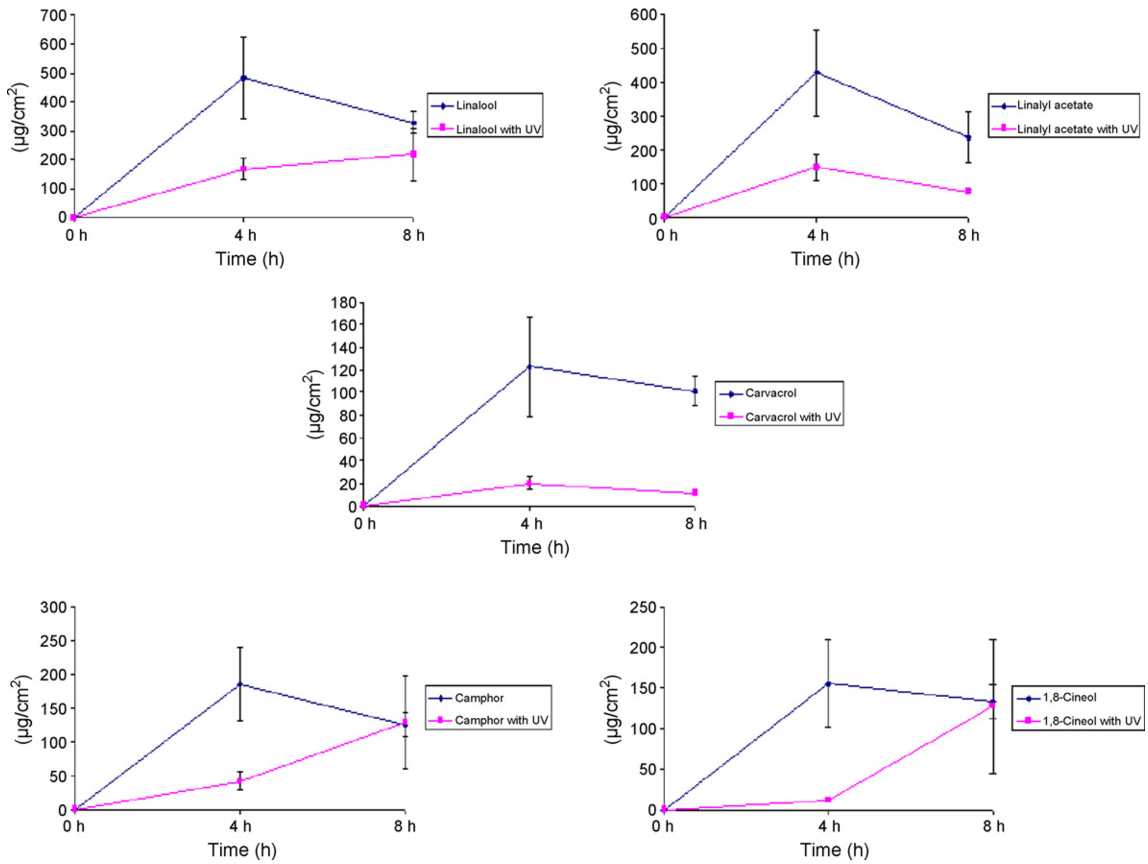

Fig. 2. Comparison between the amounts of terpenes absorbed through SC and the relation to the duration of absorption with or without UVAI radiation.

ence of polar group in the molecule could increase affinity to the polar region of stratum corneum. Indeed, the stratum corneum consist of dead, flattened cells, filled with keratine which represents the hydrophilic pathways and a lipid in intercellular spaces made of fatty acids, ceramides, and cholesterol (esters) and are arranged in bilayer structures which represents lipophilic pathways. The stratum corneum providing a formidable barrier for hydrophilic compounds which penetrate only slowly (Wester and Maibach, 2000). Polar and nonpolar substances are thought to diffuse through the skin by different mechanisms.

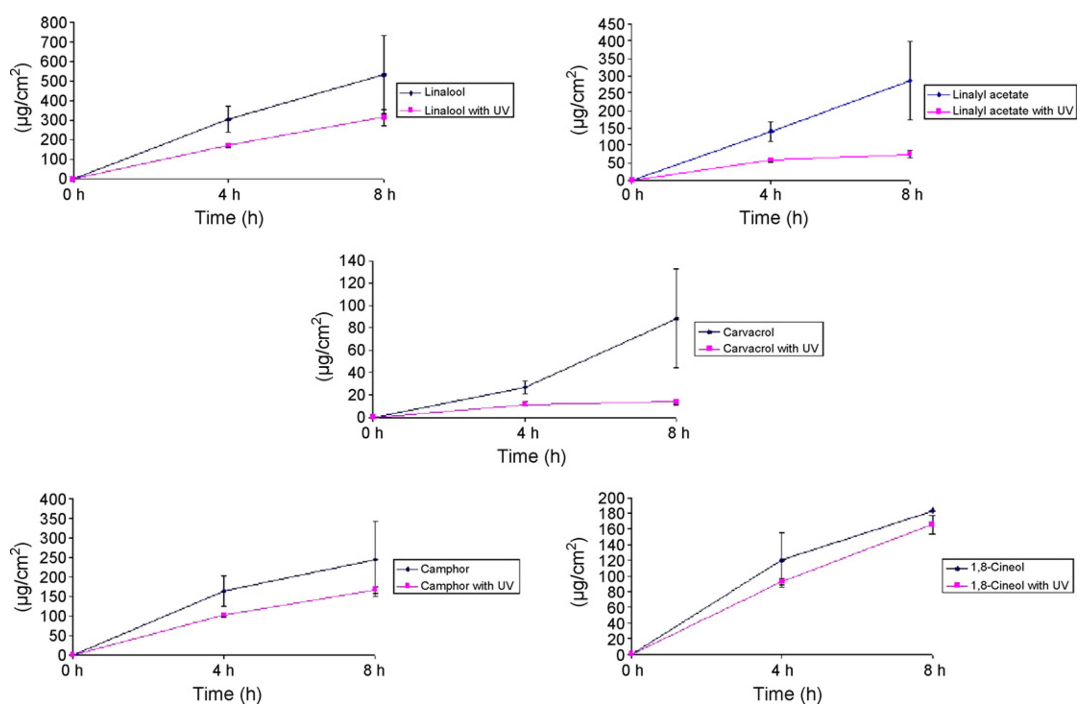

Fig. 3. Comparison between the amounts of terpenes absorbed through ED and the duration of absorption with and without UVAI radiation.

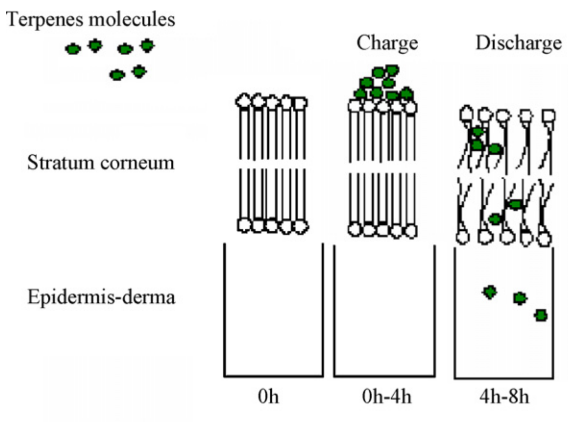

Fig. 4. Percutaneous absorption mechanism of lavender essential oil and response of the skin.

The lipid bilayers provide a continuous phase within the stratum corneum and the principal pathway by which small, uncharged molecules cross the stratum corneum (Abraham et al., 1995; Roberts et al., 1996). The pathway for a molecule by this route is greater than the thickness of the stratum corneum since it involves movement between corneocytes.

During all experiments, the terpenes were accumulated with a large quantity in ED with different speeds. However, the penetration process into these layers increased with the time of exposure. No terpenes were detected in the acceptor fluid during the absorption time. The absence of terpenes in the acceptor fluid could be explained by its high affinity for the skin and its large partition coefficient skin/physiological liquid.

According to the percutaneous absorption profile of lavender oil main compounds, we could highlight the reservoir capacity of stratum corneum for essential oil and its drainage via the deeper skin layers (epidermis-derme). This reservoir capacity includes two phases; the first was the charge phase (loading) corresponded to the accumulation of terpenes on the level of stratum corneum between the 0 and 4 h, the second was the discharge phase (drain) characterised by the increase of terpenes amount in epidermis-derma during 4–8 h. In fact, it was reported that terpenes interacted with the intercellular lipids and perturbing their lamellar packing (Cal et al., 2006) thus allowing its diffusion from stratum corneum to epidermis-derma. During this time, the deeper skin layers are charging.

The reservoir capacity (charge/discharge) allowed a prolonged release of molecules from stratum corneum to epidermis-derma; this is shown by the shape of the curves (a) and (b) (Fig. 1). This reservoir effect of stratum corneum for epidermis–dermis is shown by the linear correlation between the amount of terpenes in stratum corneum and epidermis-derma from 4 to 8 h. Between 12 and 24 h of percutaneous absorption, a new increase of terpenes amounts in stratum corneum. This time hided another cycle of charge and discharge which each lasted 4 h (12–16 h recharge, 16–20 h discharge and 20–24 h recharge). To check this alternation of phase, it was necessary to determine the molecules amount following 16 and 20 h of absorption.

The entry in a new filling phase is ensured by the return of stratum corneum lipids in its initial state of order allowing a new charge phase.

According to our investigation, we proposed a model that explains the percutaneous absorption mechanism of lavender essential oil and the response of the skin (stratum corneum and epidermis-derma) (Fig. 4).

However, in spite of the good diffusion of essential oil through the skin, the duration of stratum corneum reservoir effect for this substance is short compared to other molecules. A study carried out by Jacobi et al. (2005) on the strarum corneum reservoir effect of porcine skin for flufenamic acid showed that the effect lasts until 21 h.

In the third part of our study we have studied the influence of ultraviolet radiation (366 nm during 4 h and 8 h) on the rat skin absorption of lavender essential oil. For lower lipophilic molecules such 1,8-cineol and Camphor, the ultraviolet radiation caused a increase of charge time. According to these observations, we can report that the ultraviolet radiation caused the lengthening of the charge time (8 h instead of 4 h) by reinforcement of stratum corneum lipid bilayers making the structure more stable and the barrier tighter.

Interestingly, UVAI radiation does not disturb the cycle charge/discharge for lipophilic compounds such as Linalool, carvacrol and linayl acetate. However, we noted a drop of amplitude for both phases; 65.02% for Linalool, 65.64% for linalyl acetate and 83.35% for carvacrol. Thus, the influence of UVAI radiation on the percutaneous absorption of essential oil main compounds was different and it is a function of lipophilicity parameter. It was also probable that UVAI could act on the structure of terpene molecule and on its alignment or its rearrangement in the level of lipid bilayers. To check these hypotheses, it seems necessary to us to supplement these experiments by an exposure of oil essential only with the UVAI and an exposure of the skin only with this radiation prior to continuing the kinetic percutaneous absorption.

The lengthening of the charge phase for terpenes with weak lipophilic and the notable drop of charge/discharge amplitude for terpene with high lipophilic in the level of stratum corneum influence its accumulation amplitude into epidermis-derma.

We observed a decrease of the accumulation speed of terpenes in epidermis–dermis testifying to advantage the reinforcement of the stratum corneum stability specially the lipidic bilayers. The UVAI radiation does not modify the terpenes affinity for the epidermis-derma. This was shown by its absence in the acceptor fluid.

We reported for the first time, as far as we know, the influence of ultraviolet radiation on the kinetic absorption of lavender oil and the existence of the charge/discharge cycle in rat skin.

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