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Innovative formulations for the delivery of levothyroxine to the skin

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ABSTRACT

The aim of this work was to realize innovative transdermal formulations containing sodium levothyroxine in view of topical administration. Permeation experiments were performed in vitro, using rabbit ear skin as barrier. At the end of the permeation experiments levothyroxine retained in the skin was extracted and quantified by HPLC. Formulations tested were microemulsions and transdermal films. Microemulsions containing isopropyl myristate and isobutanol were shown to be able to increase levothyroxine solubility by the inclusion in reverse micelles. However, the inclusion in reversed micelles reduced the drug release to a significant extent, and consequently skin retention, compared to aqueous solutions. When the microemulsion was included in the transdermal film, drug retention was increased, probably for the enhancer effect of its excipients. The transdermal film proposed in this work could be an interesting alternative to semisolid formulations for the ease of use and the control in the amount of active applied. Additional benefit can be obtained if the film is used in occlusive conditions.

1. Introduction

Thyroid hormones are key regulators of metabolism and development, and are known to produce effects in many organs (Boelaert et al., 2005). Levothyroxine (T4) is the main product of thyroid secretion, together with triiodothyronine (T3).

Thyroid hormones are used topically for their activity on the skin, in particular on lipid degradation, to reduce deposits of subcutaneous adipose tissue (Pucci et al., 2000). The main effects on lipid metabolism are increased utilization of lipids, increase in the synthesis and mobilization of triglycerides stored in adipose tissue, increase in the concentration of non-esterified fatty acids and increase in lipoprotein-lipase activity. In a clinical trial, levothyroxin was found to be effective against edemato-degenerative sclerotic cellulitis and, to a minor extent, against periphlebitis (Lisi, 1973). Additionally, it has been reported that topical T3 accelerates wound healing in mice (Safer et al., 2005) and improves epidermal thickness and hair growth in rats (Safer et al., 2001) and humans (Messenger, 2000).

In a recent report (Padula et al., 2008), we characterized the permeation properties of levothyroxine across rabbit ear skin. Levothyroxine was found to be able to enter the skin but not to cross it, the main barrier to transport being the stratum corneum. Additionally, the use of occlusive conditions improved skin retention of levothyroxine, without the risk of systemic exposure, because the drug was not found in the receptor compartment. These data were obtained using a solution as formulation.

The aim of this work was to realize innovative transdermal formulations containing sodium levothyroxine in view of topical administration. The formulations tested were a microemulsion and a bioadhesive transdermal film developed in our laboratory (Padula et al., 2003; Nicoli et al., 2004; Aversa et al., 2005).

Rabbit ear skin was used as barrier in in vitro permeation experiments, because it has been shown to be a reasonable model for human skin in vitro in passive conditions (Artusi et al., 2004; Nicoli et al., 2008) and during transdermal iontophoresis (Nicoli et al., 2003, 2001).

2. Materials and methods

2.1. Materials

Levothyroxine sodium pentahydrate (T4, m.w. 888.93) was obtained from Farmalabor (Milan, I). Polyvinyl alcohol 83400 (PVA) was obtained from Nippon Ghosei (Osaka, J) and polyethylene glycol 400 (PEG400) was purchased from Fluka Chemie (Buches, CH). Span® 20 and Tween® 80 were obtained from Sigma–Aldrich Co. (St. Louis, USA). Silicone membranes (thickness 0.25 mm) were obtained from Perouse Plastie (Bornel, F). Transcutol® and polyvinyl pyrrolidone K90 (PVP) were gifts from Gattefossé Italia (Milan, I) and BASF (Ludwigshafen, D), respectively. Dimethyl-ß-cyclodextrin (DMßCD) was purchased from Wacker-Chemie (Burghansen, D).

All other reagents were of analytical grade.

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Table 1
Transdermal film composition (%, w/w on wet mass).

	PnP	PnP2	PnPME	PnPME2
PVA[a]	62.00	62.00	55.94	55.94
PVP[b]	27.00	27.00	24.36	24.36
Sorbitol[c]	4.00	4.00	3.61	3.61
Water	6.90	6.25	6.32	6.07
T4	0.10	0.75	0.75	1.00
Microemulsion	–	–	9.02	9.02
T4 content in the dried products				
μg/cm^2	19 ± 1	181 ± 23	169 ± 8	226 ± 11
% (w/w)	0.2 ± 0.0	2.3 ± 0.3	1.7 ± 0.1	2.4 ± 0.1

[a] 20% (w/w) water solution.
[b] 21% (w/w) in water:PEG400 (85:15, w/w) solution.
[c] 70% (w/w) water solution.

Methanolic sodium hydroxide was prepared by dissolving sodium hydroxide (400 mg) in methanol:water (50:50, v/v) (1000 ml), according to the USP 29.

Rabbit ears were collected immediately after sacrifice from a local slaughterhouse. The skin from the inner face was excised from the ear using a surgical blade and used immediately. The average thickness of the skin was 0.28 ± 0.06 mm.

2.2. Microemulsion preparation

The w/o microemulsions (Nandi et al., 2003; Aversa et al., 2005) were prepared by simple mixing. Briefly, isopropyl myristate (IPM) (36.3%, w/w) and isobutanol (4.5%, w/w) were mixed, then Tween® 80 (20.5%, w/w), Span® 20 (20.5%, w/w) and water (18.2%, w/w) were added. Finally, T4 was added to the microemulsion to achieve a final content of 0.75, 1.0 or 1.5 (which corresponds to the solubility of T4 in the external phase of the microemulsion) (% w/w), respectively.

2.3. Film preparation

Films containing different concentrations of T4 were prepared using the lamination technique, according to Colombo et al. (2002). The compositions of the mixtures used are reported in Table 1.

PVA was hydrated in the appropriate volume of water to a final concentration of 20% (w/w). The mixture was slowly stirred overnight using a magnetic stirrer and then heated at 90 °C to solubilize the polymer. PVP (21%, w/w) was dissolved in a mixture of PEG400:water (15:85, w/w); the appropriate amount of T4 and sorbitol 70% (w/v) were then mixed and this solution was poured into the solution of PVA under continuous stirring. When the microemulsion containing film was prepared, T4 was dissolved in the microemulsion and then added to the final mixture.

All mixtures were laminated on siliconized paper using a film-casting knife (BYK Gardner, Silverspring, MD, USA – gap 600 μm) and dried at room temperature in the dark for 24 h. After drying (final water content of 15%, w/w approximately), the films (10 cm × 20 cm) were covered with a second siliconized paper and individually sealed in aluminum pouches.

2.4. Film characterization

Once dried, 3 circles 26 mm in diameter were cut from each film. Each circle was measured for weight and thickness (Absolute Digimatic 547–401, Mitutoyo, Milan, I, resolution 0.001 mm) and then was dissolved in methanolic sodium hydroxide:HPLC mobile phase (10:90, v/v). The solutions obtained were analyzed by HPLC in order to determine the amount of T4 contained in the transdermal film. The results were expressed as % of T4 (w/w) and as μg/cm^2 (see Table 1).

2.5. In vitro transport experiments

Transdermal permeation of T4 was investigated at room temperature for stability reasons (Padula et al., 2008). Experiments were performed using vertical Franz type diffusion cells (DISA, Milan, I) with 0.6 cm^2 of diffusion area. The full thickness rabbit ear skin was mounted on the cells with the stratum corneum facing the donor compartment. The receptor compartment (4.2 ml volume) was filled with saline solution containing 1% of dimethyl-β-cyclodextrin (DMβCD), to increase T4 solubility and then to guarantee sink conditions. Two types of formulations were used in the donor compartment, microemulsions or transdermal films. The microemulsions, containing T4 at concentrations of 0.75, 1.0 and 1.5% (w/w), were applied in infinite dose conditions (0.8 ml/cm^2). The oily phase of the 0.75% (w/w) microemulsion, composed of all components except water, was tested as well. When iontophoretic pretreatment was used, the donor compartment was filled with 1 ml of saline solution and anodal iontophoresis (0.5 mA/cm^2) was applied for 30 min. Then, the saline was removed and replaced by the 1.5% (w/w) microemulsion and the experiment was continued as indicated before.

Four different films (T4 concentration in the dried product varied between 0.20 and 2.4%, w/w) were tested. The application procedure of the film on the skin surface has been previously described by Padula et al. (2003). In particular, since the film is not self-adhesive, the skin was pre-wetted with 15 μl/cm^2 of water before film application. The films were applied both in non-occlusive and occlusive conditions. In the latter, a polyethylene film was applied on the surface of the film.

At the end of the experiment (24 h) the receptor solution was sampled and analyzed by HPLC for the determination of T4 permeated. The skin was washed, weighted and extracted (30 min at 40 °C) with 1 ml of a mixture of 10 volumes of methanolic sodium hydroxide and 90 volumes of water/acetonitrile/phosphoric acid (70:30:0.1, v/v/v). This method has been shown (Padula et al., 2008) to recover more than 90% of levothyroxine contained in the skin.

The amounts of T4 recovered in the skin at the end of 24 h were normalized by the weight of tissue.

In a separate set of experiments, the permeation was studied also across silicone (LP 500-3, PerousePlastie, Bornel, France) and Cuprophan® membranes (ENKA Ag, Wuppertal, Germany). The latter were soaked in boiling water for 20 min before mounting them on diffusion cells. Samples of receptor solution were withdrawn at regular time intervals and replaced with fresh solution. The samples were analyzed by HPLC to determine the amount of T4 permeated.

The permeation profiles were fitted to Eq. (1) (Moser et al., 2001):

$$Q = (KH)C_{\text{veh}}\left[\frac{D}{H^2}t - \frac{1}{6} - \frac{2}{\pi^2}\sum_{n=1}^{1}\frac{(-1^n)}{n^2}\exp\left(\frac{-Dn^2\pi^2 t}{H^2}\right)\right] \quad (1)$$

where Q is the cumulative amount of T4 permeated per unit area at time t, C_{veh} is the concentration of the drug in the donor vehicle, K is the stratum corneum/vehicle partition coefficient, D the diffusion coefficient and H the diffusion path-length. The permeability coefficient P was calculated as the product between KH and D/H^2. The fitting was performed using KaleidaGraph® 4.0 (Synergy Software) running on a MacIntosh MacBook Pro.

2.6. Analytical method

The amount of T4 in the samples was quantified by high performance liquid chromatography (HPLC) as reported previously (Padula et al., 2008). Briefly, the instrument was a PerkinElmer liquid chromatograph (PerkinElmer, Norwalk, CT, USA) which included a UV detector, set to 225 nm and an analytical Spherisorb®

Cyano column (2.1 mm × 250 mm), from Waters (Millipore Corporation, Milford, USA). A mixture of water/acetonitrile/phosphoric acid (70:30:0.1, v/v/v) was used as mobile phase, at a flow rate of 0.3 ml/min. Injection volume was 20 μl. The limit of detection was 0.001 μg/ml and the limit of quantification was 0.09 μg/ml (Padula et al., 2008).

2.7. Dynamic Light Scattering (DLS) analysis

Dynamic light scattering (DLS) analysis was performed at 25 °C on both the microemulsion and the oily phase of the microemulsion, using a 90Plus apparatus (Brookhaven instruments Corporation, Holtsville, NY, USA). The intensity autocorrelation function was measured at 90° during 2 min. The viscosity value of the sample (90 cP) used for the calculation was determined using a rotational viscosimeter.

2.8. Statistical analysis

Each experiment was replicated at least 4 times. The significance of the differences between values was assessed using ANOVA (KaleidaGraph® 4.0 software on a Macintosh MacBook Pro) followed by Bonferroni's test.

3. Results and discussion

Based on our previous experience (Padula et al., 2008), the experiments were performed at room temperature using freshly excised rabbit ear skin as barrier, to guarantee the stability of T4 throughout the experiment.

For all the formulations tested on the skin, T4 was never found in the receptor compartment, while it was found in the skin. Owing to the sensitivity of the analytical method, fluxes lower than 10 ng cm^{-2} h^{-1} would not be detectable. The amounts of T4 recovered in the skin after 24 h were normalized by the weight of tissue, to calculate the concentration of the hormone in the tissue, expressed as nanograms of T4 per milligram of tissue.

Two formulations types were studied, microemulsions and films.

3.1. Microemulsion

Microemulsions have been shown to be efficient formulations for the transdermal and dermal delivery of – particularly – lipophilic compounds (Kreilgaard, 2002; Santos et al., 2008), because of their solubilizing properties and also because their components may act as penetration enhancers. The w/o microemulsion used in the present work contains isopropyl myristate, isobutanol, Tween® 80, Span® 20 and water; this formulation has been shown to increase progesterone solubility and flux across the skin (Aversa et al., 2005). Because the lipophilicity of T4 is very high (log *P* 7.36 (Kasim et al., 2004)), the drug was dissolved in the oily phase of the microemulsion. Fig. 1 reports the concentration of T4 retained in the skin after 24 h of application of the microemulsions containing 0.75, 1.0 and 1.5% of T4, in infinite dose conditions. T4 skin concentration increased slightly with drug concentration, although the difference was statistically significant ($p < 0.05$) only between 0.75 and 1.5% microemulsions.

Skin pretreatment with iontophoresis (anodal iontophoresis, 0.5 mA/cm^2 for 60 min) and subsequent application of the 1.5% microemulsion (Fig. 1) produced an increase in skin concentration ($p < 0.05$), in analogy with what was observed in our earlier work (Padula et al., 2008) with the commercial cream.

The different microemulsions tested did not produce any permeation across the skin. To try to elucidate the effect of the vehicle on T4 release and transport, the same formulations were also tested

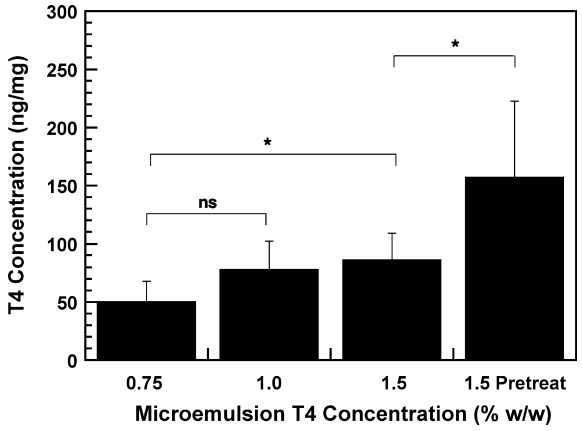

Fig. 1. Levothyroxine retained in the skin after 24 h of application of microemulsions containing different T4 concentrations. Pretreatment refers to iontophoresis (60 min, 0.5 mA/cm^2) applied before formulation application. Average values ± SD. Significantly different: $^*p < 0.05$.

across an artificial membrane. Preliminary experiments across silicone membranes produced no detectable amounts of T4 in the receptor solution for any of the formulations tested (meaning fluxes lower than 10 ng cm^{-2} h^{-1}), indicating a very low permeability of the molecule. For this reason, a dialysis membrane (Cuprophan®) was used.

Fig. 2 reports the permeation profiles obtained using a Cuprophan® membrane as barrier, from the formulations previously tested across the skin. The permeation profiles of T4 were linear with time after a short lag time and were very similar among them, in analogy with the skin retention data (Table 2). Saturated aqueous formulations of T4 were tested as well, to evidentiate the role of microemulsion components on T4 transport. Since cyclodextrins are known as solubilizing agents for water insoluble compounds and, in particular, DMβCD has been shown to increase T4 solubility up to 20 times (Padula et al., 2008), the experiments were performed also using this molecule a solubilizing agent for T4. The results obtained, using a saturated solution in the presence or absence of 200 mM DMβCD, are reported in Fig. 2. The highest profile was obtained from a solution of T4 in water containing DMβCD, followed by neat water solution and by the microemulsions. The corresponding skin retention data previously obtained (Padula et al., 2008) follow the same trend (see Table 2). This result is not completely justified by differences of solubility/concentration of T4 in the different formulations. In fact, the

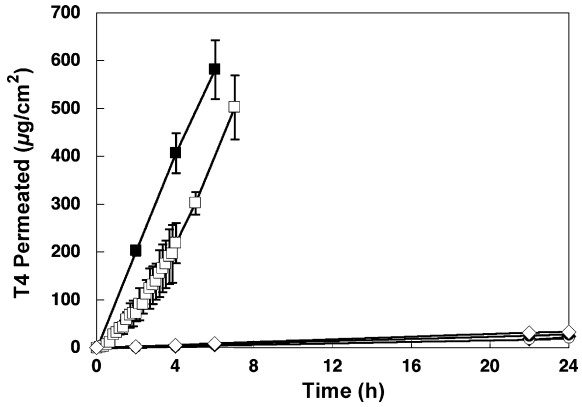

Fig. 2. Permeation profiles of levothyroxine across Cuprophan® membranes, from microemulsions containing 0.75 (○), 1.00 (●) or 1.50 (◊) % (w/w) of T4, from the oily phase of the microemulsion (♦) and from a saturated water solution, in the presence (■) and absence of 200 mM DMßCD (□). Average values ± SD.

Table 2
Cumulative amounts recovered in the skin after 24 h and Cuprophan® membrane permeation parameters of T4 from different formulations (mean values ± SD).

Formulation	Drug concentration (mg/ml)	Skin retention (ng/mg)	$KH \times 10^3$ (cm)	D/H^2 (h^{-1})	Permeability coefficient (cm h^{-1}) $\times 10^4$	Limiting flux ($\mu g\,cm^{-2}\,h^{-1}$)
Water	3.0	187 ± 100[a]	70.87 ± 22.61	0.29 ± 0.03	202.9 ± 49.6	60.9 ± 14.9
DMßCD	11.7	134 ± 94[a]	28.5 ± 11.5***	0.37 ± 0.13	94.6 ± 10.5***	110.7 ± 12.3***
Microemulsion	7.5	50 ± 17**	0.85 ± 0.36***	0.20 ± 0.07	1.6 ± 0.3***	1.2 ± 0.2***
Microemulsion	10.0	78 ± 27*	0.83 ± 0.63***	0.15 ± 0.04	0.9 ± 0.8***	0.9 ± 0.8***
Microemulsion	15.0	86 ± 23*	0.67 ± 0.39***	0.19 ± 0.06	1.1 ± 0.3***	1.6 ± 0.5***
Oily phase	9.0	n.d.	0.86 ± 0.34***	0.14 ± 0.02	1.2 ± 0.4***	0.9 ± 0.3***

n.d. = not determined.
[a] From reference (Padula et al., 2008).
* $p < 0.05$, significantly different from water solution.
** $p < 0.01$, significantly different from water solution.
*** $p < 0.001$, significantly different from water solution.

solution of DMβCD and the 1.5% (w/w) microemulsion gave rise to profiles (and skin retention data) largely different, even though they were both saturated with T4 and the solubility of the hormone is comparable (15 mg/ml vs 11.7 mg/ml).

In order to explain these differences, the relevant permeation parameters were calculated by fitting the permeation profiles to Eq. (1). Relevant permeation parameters are reported in Table 2: the partitioning parameter (KH) gives an indication of the partitioning of the molecule between the barrier and the donor solution and the diffusive parameter (D/H^2) describes the diffusion of the permeant across the barrier.

The value of the diffusive parameter was similar for all formulations tested, indicating that the different solvent/excipients did not influence the diffusion of T4 across the membrane. On the contrary, the partitioning parameter changed according to the formulation used, and in particular it was two orders of magnitude higher for water solution than for the microemulsions. Additionally, the neat water solution showed a KH value significantly higher than that obtained with DMβCD, probably because of the reduced concentration of free T4 in the presence of the complexing agent. The differences observed in the value of the partitioning parameter were observed also in the permeability coefficient and in the flux values.

From these results it appears that the differences in performance among aqueous formulations and microemulsions are due to differences in partitioning.

To try to understand the reasons why the three microemulsions gave such low performances compared to aqueous solutions, the components of the microemulsion were isolated and tested separately. Firstly, the oily phase of the microemulsion (IPM, isobutanol and surfactants) was used as donor formulation: no differences with the corresponding microemulsion were observed (see Fig. 2 and Table 2). Then, a solution of T4 in IPM/isobutanol was prepared, and a dramatic reduction of solubility was observed (0.2 mg/ml vs 15 mg/ml). This solubility decrease is evidently due to the absence of surfactants (Tween® 80 and Span® 20) that are probably capable of interacting and solubilizing T4. One possibility is that in the organic solution of IPM, the surfactants (Tween® 80 and Span® 20) form reversed micelles, containing isobutanol in their interior. Reverse micelles are aggregates of surfactants in oily systems, in which the lipophilic part of the surfactant molecule faces the oily medium whilst the hydrophilic part faces the core which can include a small amount of polar solvent (Muller-goymann, 2004). In this specific case, Tween® 80 and Span® 20 represent the nonionic surfactants, IPM the oily phase and isobutanol the polar solvent. The inclusion of T4 into reversed micelles then reduces the amount of drug available for permeation across the membrane. DLS analysis of the oily phase of the microemulsion demonstrated the presence of aggregates of 2.8 ± 0.2 nm, a size compatible with that of reverse micelles. When water was present, the particle size increased to 11.1 ± 2.4 nm, probably due to the incorporation of water in the reverse micelles and consequent swelling of the aggregates.

These results support the formation, in the microemulsion systems, of reversed micelles able to solubilize T4, thus reducing the amount of free drug available for permeation across the membrane. The performance of the microemulsions with different drug loading was comparable because the concentration of free T4 was constant.

3.2. Film

The use of a semisolid formulation, such as a lotion or a cream, leads to some disadvantages such as the uncertainty of the dose applied and of the contact time. For these reasons, a new transdermal drug delivery system (Colombo et al., 2002; Nicoli et al., 2005; Nicoli et al., 2004) was prepared and tested. The composition of the films prepared is reported in Table 1. This new transdermal delivery system is a bioadhesive film very thin, transparent, flexible, mechanically resistant and permeable to water vapor (Padula et al., 2005). The film, which includes the backing, the adhesive and the drug-reservoir functions in one layer of material, is not adhesive in the dry state but bioadhesive when applied on wet skin. The results of skin retention from films are reported in Fig. 3. The first film prepared, PnP, with a drug loading of 0.2% (w/w), did not produce any T4 accumulation into the skin. When drug loading was increased to 2.3% (PnP2) modest amounts of T4 were found in the skin. Owing to the good result, in terms of T4 solubility and skin accumulation obtained with the microemulsion (see Fig. 1), the microemulsion was included in the formulation of the film (see Table 1). Two different films with drug loadings of 1.7 (PnPME) and 2.4 (PnPME2) % (w/w) were prepared and tested. The resulting skin accumula-

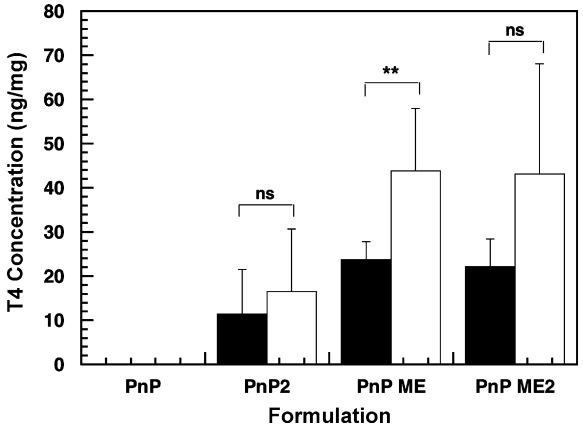

Fig. 3. Levothyroxine retained in the skin after 24 h of application of film with different T4 concentration, in occlusive (white bars) and non-occlusive (black bars) conditions. Average values ± SD. Significantly different: **$p < 0.01$.

tions, reported in Fig. 3, were similar between them but higher than those obtained from film PnP2. In particular, comparing films with the same loading (PnP2 vs PnPME2), it appears that the presence of the microemulsion in the formulation improved T4 skin retention, probably for the enhancing effect of surfactants or isopropyl myristate. All formulations tested in occlusive conditions gave superior results compared to the same formulation in non-occlusive condition, although the difference was statistically significant only for PnPME film.

4. Conclusions

The results obtained in the present work support the formation, in the microemulsion system, of reversed micelles able to solubilize T4, thus reducing the amount of free drug available for permeation into the skin. The performance of microemulsions with different drug loadings was comparable because the concentration of free T4 was constant. The microemulsion could be included in a bioadhesive transdermal film, studied as innovative formulation for T4, and the result was increased skin retention, probably produced by isopropyl myristate and/or the surfactants present. The transdermal film proposed in this work could be an interesting alternative to semisolid formulations for the ease of use and the control in the amount of active applied. An additional improvement could be obtained when the film was used in occlusive conditions. However, this formulation can still be improved, for instance by using chemical or physical penetration enhancers, and this will be the subject of future investigations.

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