Release and skin permeation of lapachol from microemulsion and gel dosage forms

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INTRODUCTION AND OBJECTIVE

Lapachol (4-Hydroxy-3-(3-methylbut-2-enyl) naphthalene-1,2-dione) is a naftoquinone isolated from many species of Bignoniaceae found in Brazil, which features significant anti-inflammatory activity (Santana 2008).

The anti-inflammatory therapy aims either antagonization or blocking of the main proinflammatory mediators. However, many of these agents produce serious adverse reactions. Therefore, it is of fundamental importance the development of topical pharmaceuticals (Hussain 2007).

The low aqueous solubility of lapachol limits its formulation as a transdermal dosage form, making valuable the incorporation of this drug in microemulsion systems, which act increasing the efficacy of drugs, allowing reduction of the dose administered and minimizing side effects.

To ensure the effectiveness of these systems, we evaluated the release and permeation profiles.

MATERIALS AND METHODS

It was prepared three (3) different microemulsions (ME) and a gel (Table 1), which were developed by our research group. The gel was prepared by dispersing the polymer in purified water by mechanical stirring. The ME was obtained by blending specific proportions of surfactant/ cosurfactant (Cremophor EL® and Tween 20®) to oil phase (oleic acid) and water. The lapachol (0.5%) was incorporated into the oily phase. The mixture was homogenized by stirring by Ultra-Turrax ®.

The *in vitro* release test was performed using Franz diffusion cells with artificial membrane of cellulose acetate. The receptor solution consisted of phosphate buffer (pH 7.4), polyoxyethelene (20) and oleyl ether (5%). The collect of the samples were made at specific times: 0.5h, 1.0h, 1.5h, 2.0h, 2.5h, 3.0h, 4.0h, 6.0h, 8.0h, 10.0h and 12.0h. The analysis of the released lapachol amount was performed by UV spectroscopy.

The skin permeation test was evaluated under the same conditions of the release study, however, the membrane used was pig ear skin and the collection times were: 2, 4, 6, 10, 12 and 24 hours. The analyzes were performed by HPLC/UV at a wavelength of 273 nm using, as mobile phase, methanol and aqueous 5% acetic acid (80:20).

Table 1 : Qualitative and quantitative composition of the obtained dosage forms

Composition (%)	ME 1	ME 2	ME 3	GEL
Cremophor®	27.1	41.6	8.4	-
Tween 20®	5.4	8.4	41.6	-
Oleic Acid	31.9	25.0	25.9	-
Carbopol Ultrex®	-	-	-	0.5
Tween 80®	-	-	-	0.025
Triethanolamine	-	-	-	pH8.0
Ethanol	-	-	-	28
Glycerin	-	-	-	12
Lapachol	0.5	0.5	0.5	0.5
Water q.s.p.	100	100	100	100

RESULTS AND DISCUSSION

The ME and gel release profiles are shown in Figure 1.

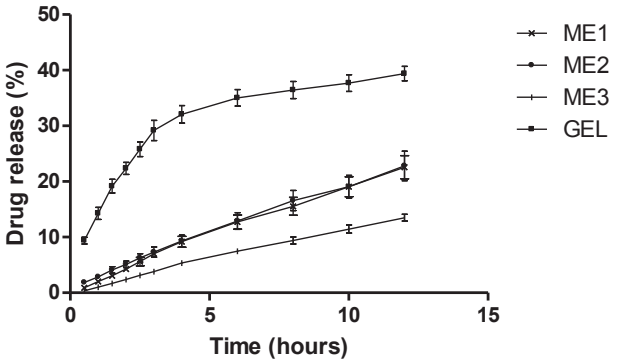

Figure 1: ME and gel *in vitro* release profile.

The cumulative amount of lapachol released after 12h and its release rate (flow through a cellulose membrane) decreased in the following order: Gel > 1 ≈ 2 > 3 (Table 2) .

Table 2: Cumulative amount released after 12h (Q_{12} ± S.D.), Flux ± S.D. of lapachol from micromulsions (MEs) 1–3 and gel.

Formulation	Q_{12} ± S.D. ($\mu g\ cm^{-2}$)	Flux ($\mu g\ cm^{-2}\ h^{-1}$)
ME 1	338.50 ± 31.29	24.31
ME 2	341.90 ± 39.87	23.38
ME 3	202.41 ± 8.84	17.11
GEL	679.43 ± 22.27	34.11

After 12h, ME 1 and 2 had flow rate and released quantities statistically similar (p> 0,05) (ANOVA, two-way). However, the others formulations differed, statistically, almost since the beginning (ME 1 *vs* ME 2 since 2.0h; ME 2 *vs* ME 3, 1.5h; and EMs *vs* GEL differed in all collection times).

The model which best fitted for the MEs was the zero order one, as it showed the highest determination coefficient. This model suggests that the release velocity is constant, being independent of drug concentration. This constant speed is highly desirable in drug delivery. However, the gel's behavior showed to be of pseudo-first order (Higuchi), i.e., the diffusion is controlled by the system.

A higher concentration of Cremophor EL®, compared to the Tween 20®, favored the release of lapachol. Probably, this happened because the lapachol solubility is higher in Tween 20® than in Cremophor EL®. The gel showed a greater amount released at the end of 24 h, compared with the MEs formulations due to the low solubility of lapachol in water (Torelli-Souza 2012).

The results of the in vitro permeation tests for all the formulations are reported in table 3 and figure 2. In the first 12 hours of the experiment, there was no difference between the formulations. However, after 12 hours, we can notice that the 3 MEs have statistical differences ($p<0.05$), when compared to the gel (ANOVA, two-way).

As shown in Table 3 and Figure 2, it is clear that the formulation gelled presented a greater amount permeated when compared to microemulsions. This is probably due to the fact that this formulation presents a large amount of aqueous phase. Furthermore, the gel formulation contains alcohol, which acts as permeation promoter (Mainarde 2010).

Comparing the in vitro release results with the data of the permeation through the skin, it was observed that the amount of lapachol released from the vehicle was greater than the permeate for all formulations tested. This suggests that the limiting step of the permeation rate of lapachol was its passing through the skin, instead its releasing from the vehicle.

The literature reports that microemulsions have great capacity to promote the penetration of drugs through the skin. However, in this study, it was obtained a lower amount of permeated lapachol and a lower flow rate for the MEs, when comparing to the gel.

Table 3: Cumulative amount released after 12h (Q_{12} ± S.D.), Flux ± S.D. of lapachol from micromulsions (MEs) 1–3 and gel

Formulation	Q_{24} ± S.D. (µg cm^{-2})	Flux (µg cm^{-2} h^{-1})	Lag time (h)
ME 1	5.02 ± 2.56	0.22	0.58
ME 2	6.54 ± 1.80	0.27	0.75
ME 3	2.69 ± 1.89	0.11	0.25
GEL	56.06 ± 36.30	2.31	9.00

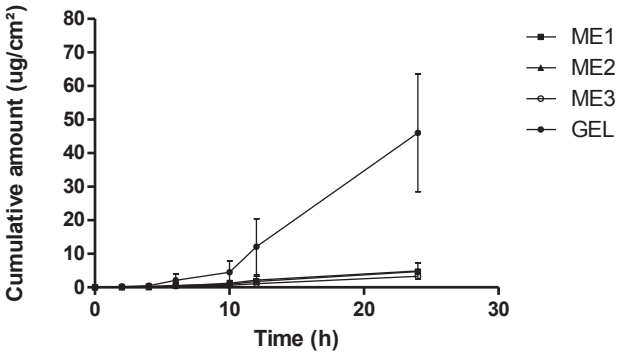

Figure 2: *In vitro* permeation profile of the formulations studied.

CONCLUSIONS

Gel formulation provided a significantly higher skin permeated (about 10 fold) amount of lapachol as compared to microemulsions. Hence, these results suggest that the topical delivery of lapachol from gel formulations can be an effective medication for topical injuries.

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