

## Evaluation of copaiba oil as enhancer of ibuprofen skin permeation

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The administration of medications on the skin through transcutaneous routes is a practice that has been used by mankind for millennia. Some studies have been reporting the use of terpenes and natural oils rich in terpenes as an enhancer of cutaneous penetration. Copaiba oil, due to its rich content of terpenes, presents itself as a great choice of penetration enhancer for drugs administered on the skin. In this study, we developed two cream formulations containing 5% of ibuprofen (IBU) and copaiba oil: IBCO5 and IBCO10 with 5% and 10% of copaiba oil respectively. *Ex vivo* cutaneous penetration/permeation studies of IBU were performed using pig ear skin as biological membrane in the Franz-type diffusion cells. The steady-state flux of IBU samples, IBCO5 ( $35.72 \pm 6.35$ ) and IBCO10 ( $29.78 \pm 2.41$ ) were significantly higher when compared with control without copaiba oil ( $10.32 \pm 1.52$ ) and with a commercial product ( $14.44 \pm 2.39$ ). In the penetration analysis, the amount of IBU found in the samples IBCO5 and IBCO10 was markedly higher in the dermis than epidermis. Our results showed that copaiba oil possesses attracting properties in promoting skin penetration and permeation of IBU when added into cream formulations.

**Keywords:** Copaiba oil. Terpenes. Ibuprofen. Penetration enhancer. Pig skin.

### INTRODUCTION

Transcutaneous formulations are an interesting approach to transport several drugs, as an alternative to overcome issues related to the pharmacokinetics and pharmacodynamics when drugs are administered by other routes (Simon *et al.*, 2016; Hussain *et al.*, 2012). The advantages of the percutaneous delivery reside not only in reducing fluctuations in plasma drug concentrations and frequency of administration but also reducing variability of oral absorption and mitigating the pre-systemic metabolism. Furthermore, this route allows

abrupt discontinuation of treatment and constitutes an attracting alternative to intravenous administration (Obata *et al.*, 2010).

Among factors that have the potential to perturb percutaneous absorption are skin hydration state and its biological characteristics, as well as the physical and chemical properties of the drug, including its molecular mass, aqueous solubility, melting point, partition coefficient (Log P) and dissociation constant (pKa). In addition, pharmaceutical and adjuvant forms used to convey drugs could also affect drug absorption through transdermal delivery (Hussain *et al.*, 2012; Pontrelli, Monte, 2014).

The use of permeation enhancers, incorporated in different types of formulations, improve drug flux through diverse membranes. These chemicals are usually pharmacologically inactive and are able to permeate or interact with the constituents of the stratum corneum when

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incorporated into a transcutaneous formulation, reducing skin resistance to the diffusion of a drug (Jiang *et al.*, 2017). Several studies has been shown that the use of natural oils rich in terpenes as permeation enhancers are safe and suitable to promote the percutaneous absorption of drugs from topical formulation into lower skin layers (Herman, Herman, 2015).

Among natural compounds with potential applications as permeation enhancer, stands out copaiba oil. This oil is obtained by tapping the trunk of the trees from species of *Copaifera* L. (Leguminosae), popularly known as “copaiba” or “pau-de-óleo” (Oliveira *et al.*, 2010). Copaiba oil is from natural source, rich in terpenes, mainly sesquiterpenes and constitutes one of the most important renewable sources of natural remedy for population of the Amazon region and can be found in drugstores and markets all over Brazil (Leandro *et al.*, 2012). Furthermore, copaiba oil has been reported to increase skin permeation of drugs, suggesting its use as a penetration enhancer in topical dosage forms (Veiga-Junior *et al.*, 2007).

Topical drug delivery of Ibuprofen using natural oils as permeation enhancers have been reported in the literature (Chen *et al.*, 2016; Khan *et al.*, 2011). Ibuprofen is a classical non-steroidal anti-inflammatory drug (NSAID) which is used for the symptomatic treatment of rheumatoid arthritis, ankylosing spondylitis, dysmenorrhea, etc. However, an effective permeation of Ibuprofen, is difficult to achieve by transdermal delivery due to its poor skin permeability (Bello, Kuwornu, 2014; Padula, Nicoli, Santi, 2011). Thus, the purpose of the present study was to develop and evaluate a novel, simple and stable topical cream formulation containing 5% of IBU, using copaiba oil (5% and 10%) as skin permeation enhancer, in comparison with commercial cream formulation containing 5% of ibuprofen.

## MATERIAL AND METHODS

### Chemicals

Ibuprofen primary standard was purchased from United States Pharmacopeia (Rockville, MD, USA) and ibuprofen raw material from Henrifarma (São Paulo, SP, Brazil). Copaiba oil was purchased from Beraca Sabará (São Paulo, SP, Brazil) and  $\beta$ -caryophyllene from Sigma

Aldrich (St. Louis, MO, USA). VersaPro™ topical cream base was purchased from Medisca Pharmaceutique Inc (St. Laurent, QC, Canada). Dolgit® cream (Merck Serono, France) is a commercial product containing 5% IBU and it was purchased from a French drugstore in Paris. Methanol HPLC grade (Scharlau, Spain), orthophosphoric acid (Merck, Germany), potassium phosphate monobasic (Spectrum, USA), sodium hydroxide TS (Spectrum, USA).

### GC-MS of copaiba oil

Copaiba oil was analyzed by GC-MS on a Shimadzu QP2010 Plus (Shimadzu Corporation, Kyoto, Japan) system equipped with an AOC-20i auto sampler operating in the electron ionization (EI) mode at 70 eV under the following conditions: Restek Rtx-5MS fused silica capillary column (30 m x 0.25 mm i.d. x 0.25  $\mu$ m film thickness) composed of 5%-phenyl-95%-methylpolysiloxane; carrier gas helium (99.999%) at a constant flow rate of 1.46 mL/min; injected volume of 0.1  $\mu$ L of a solution containing copaiba oil (2  $\mu$ L) and standard  $\beta$ -caryophyllene (2  $\mu$ L) in 1000  $\mu$ L of hexane without flow divider; injector temperature 245 °C; ion-source temperature 280°C. The oven temperature was programmed to increase from 60 to 240 °C at 3 °C/min. Mass spectra were recorded with a scan interval of 0.5 s within the mass range 40–600 Da. Quantification of each constituent was estimated by internal normalization (%). The identification of the copaiba oil components was based on their retention indices, relative to a homologous series of *n*-alkanes (C<sub>8</sub>-C<sub>20</sub>) measured on an Rtx-5MS capillary column under the same operating conditions. Computer matching was accomplished with the aid of the Wiley 7 and NIST 08 spectra libraries (McLafferty, 2008). The mass spectra of the constituents were also compared to those reported in the literature (Adams, 2007).

### Sample preparation

VersaPro™ cream, a pre-made oil-in-water (O/W) emulsified base, was purchased in order to make a cream formulation containing: (1) ibuprofen 5% as control; (2) ibuprofen 5% and copaiba oil 5% (IBCO5) and; (3) ibuprofen 5% and copaiba oil 10% (IBCO10), where

copaiba oil was used as cutaneous penetration enhancer. This base was chosen because of its excellent stability for incorporation of water and oil-soluble drugs (Sarkar *et al.*, 2011). Finally, a commercial cream containing Ibuprofen 5% was used as reference for permeation and penetration studies as well as for the stability tests.

### Preliminary stability studies

Samples were prepared in triplicate and evaluated after 24 hours ( $t_0$ ) and 12 days ( $t_{12}$ ) of heating-cooling cycles for the following parameters: color, odor, consistency, brightness, absence of clumps and precipitates, pH analysis, lack of homogeneity after mechanical stress (centrifuge) and IBU content (Zamarioli *et al.*, 2015). This 24 hours heating-cooling cycle test on the formulation samples was performed by storing the samples in the fridge/oven ( $-5 \pm 2^\circ\text{C}$  and  $45 \pm 2^\circ\text{C}$ ) as required for 12 days. The organoleptic properties such as consistency, color, odor, glare and lack of clumps and precipitates were evaluated by direct visual perception.

The pH of the formulations was measured using a digital pH meter Model PG2000 (Gehaka, São Paulo, SP, Brazil) previously calibrated with solutions of pH 4.0 acetate and pH 7.0 phosphate buffers. 10% aqueous solution (w/v) samples were prepared and their pH was measured, in triplicate, at room temperature.

For the physical stability test, samples were placed in tubes and subjected to conditions of mechanical stress by centrifugation at a speed of 1370 g for 15 min in triplicate. Samples were evaluated visually for precipitation, coalescence and for separation of the emulsified preparations.

Additionally, ibuprofen assay was performed by liquid chromatography (HPLC), according to the method described in British Pharmacopoeia, 2014. The chromatographic system consisted of an Alliance 2695 solvent delivery module (Waters, USA) equipped with auto sampler, degasser, column oven and a dual wavelength UV-Vis detector 2489. The chromatographic conditions were: X-Bridge C18 column, 4.6 x 150 mm, 5  $\mu\text{m}$ , connected to a C18 guard column (Waters Assoc., Milford, USA); isocratic mobile phase of methanol-water (75:25, v/v) adjusted to pH 3.0 with orthophosphoric acid;

flow rate of 1.0 ml/min, injection volume of 20  $\mu\text{L}$  and wavelength of 222 nm.

### Ex vivo permeation study

For each formulation, an *ex vivo* test using Franz-type diffusion cell were carried out for the purpose of evaluating the permeation of IBU from cream formulations into the skin. Phosphate buffer (pH 7.4) was used as receptor solution and excised skin from pig ear was used as biological membrane. Pig ear skin is a very attractive model for *ex vivo* percutaneous absorption studies since it mimics human skin (Yuan, Capomacchia, 2005).

The pig ear was obtained from local slaughterhouse and the skin was excised from the dorsal part of the pig's ear using a scalpel. The hair and excess fat were removed and only skin cuts in the range of 1.5 – 2.0  $\text{cm}^2$  were used in the experiments (Lucca *et al.*, 2015). The receptor compartment was filled with 7.5 mL of phosphate buffer pH 7.4. Cells were maintained at  $37^\circ\text{C} \pm 0.5$  in a thermostatic bath under agitation (900 rpm) (Santis *et al.*, 2012). Cream samples (200 mg) were applied on the membrane by using an automatic pipette for semisolid preparations. The diffusion area of each donor compartment corresponded to approximately 1.00  $\text{cm}^2$ .

After applying samples, an aliquot of 1 mL of receptor solution was sampled each 90 min for analysis of IBU content, and the same volume was replaced with fresh buffer to maintain *sink* conditions. After that, IBU levels were analyzed by high-performance liquid chromatography (HPLC). The permeation of IBU in the receptor medium was monitored for 450 min (Herkenne *et al.*, 2007).

To calculate the amount of IBU permeated (Q) at time t, dilutions obtained after the first collection were considered, and the following equation (1) was used according to Sato *et al.* (2007):

$$Q = C_{\text{measured}} \times V_r + \sum^{n-1} C_a \times V_a \quad (1)$$

Where:

$C_{\text{measured}}$  = concentration of the sample at time t

$V_r$  = volume of receptor solution of the diffusion cell

$C_a$  = concentration of the sample removed

$V_a$  = volume of the sample removed

A permeation curve of IBU, expressed in  $\mu\text{g cm}^{-2}$  of permeated drug versus time (min), was then generated. Values obtained by HPLC were used to calculate kinetic parameters (kinetic model), latency time (lag time), steady-state flux (J) and permeability coefficient (Kp) of IBU through the membrane (Santis *et al.*, 2012; Stahl, Wohler, Kietzmann, 2011).

To determine the kinetics of permeation, Higuchi, First-order and Zero-order model kinetics were calculated. The closest linear correlation coefficient ( $R^2$ ) to 1 corresponded to the membrane permeation kinetics of IBU (Sato *et al.*, 2007).

### Penetration in the epidermis and dermis

To determine IBU penetration in the dermis and epidermis, a validated method by Santis *et al.* (2012) was used. After the permeation test, the system was dismantled and the skin was removed from the diffusion cell, and cleaned. Epidermis was separated from the dermis using a surgical scalpel, and both were subjected to the extraction process of IBU by adding 1 mL of mobile phase (methanol-water 75:25 v/v). Material was vortexed three times for 30 sec. Samples were then centrifuged at 6400 rpm for 10 min. Supernatant was collected and samples were analyzed by HPLC (Zamarioli *et al.*, 2015). As a result, a graph representing dermal and epidermal penetration of IBU was generated. Results were used to evaluate the ability of copaiba oil as a permeation enhancer of IBU through different skin structures (Santis *et al.*, 2012).

### Statistical analysis

Results are presented as mean  $\pm$  standard deviation. Statistical significance was determined by Student's t test for two group comparisons, and one-way ANOVA followed by the Newman-Keuls test for multiple comparisons through GraphPad Prism software. Statistical differences were considered significant when p-values were less than 0.05.

## RESULTS

### Chemical constitution of copaiba oil

Chromatographic analysis by gas GC-MS allowed the detection of several sesquiterpenes from the copaiba oil. The identification of these substances was performed by comparison of their mass spectra with those of the Wiley 7 and NIST 08 data libraries, as well as by comparison of their retention times obtained with already published indices (McLafferty, 2008; Adams, 2007). A total of 16 compounds were identified as following: 14 hydrocarbon sesquiterpenes (**1-14**; 97.7%) and two oxygenated sesquiterpenes (**15** and **16**; 2.4%).  $\beta$ -caryophyllene (**4**; 34.9%),  $\alpha$ -humulene (**6**; 9.9%), germacrene D (**9**; 9.3%),  $\alpha$ -bergamotene (**5**; 9.2%),  $\alpha$ -copaene (**2**; 7.7%),  $\delta$ -cadinene (**13**; 5.6%) and  $\alpha$ -muurolene (**10**; 5.2%). The results are shown in Table I.

**TABLE I** - Chemical composition of copaiba oil identified by GC-MS

Substances	RT (min)	KI (lit.)	KI (exp.)	RA (%)	SI (%)
$\delta$ -elemene (1)	13.598	1335	1339	1.19	92
$\alpha$ -copaene (2)	14.689	1374	1380	7.65	94
$\beta$ -elemene (3)	15.091	1389	1395	2.95	96
$\beta$ -caryophyllene (4)	15.976	1423	1428	34.94	97
$\alpha$ -bergamotene (5)	16.240	1432	1438	9.21	95
$\alpha$ -humulene (6)	16.794	1452	1459	9.91	95

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**TABLE I** - Chemical composition of copaiba oil identified by GC-MS

Substances	RT (min)	KI (lit.)	KI (exp.)	RA (%)	SI (%)
$\gamma$ -gurjunene (7)	16.972	1475	1465	1.53	88
$\gamma$ -amorfone (8)	17.354	1495	1480	3.53	90
germacrene D (9)	17.513	1484	1486	9.32	92
$\alpha$ -muurolene (10)	17.995	1500	1503	5.20	93
$\beta$ -bisabolene (11)	18.181	1505	1508	3.04	93
$\gamma$ -cadinene (12)	18.424	1513	1516	1.67	87
$\delta$ -cadinene (13)	18.698	1522	1524	5.62	90
germacrene B (14)	19.871	1559	1558	1.89	90
caryophyllene oxide (15)	20.837	1582	1586	0.39	83
$\alpha$ -cadinol (16)	23.490	1652	1650	1.96	89
<b>Hydrocarbon Sesquiterpenes</b>				<b>97.65</b>	
<b>Oxygenated Sesquiterpenes</b>				<b>2.35</b>	

RT: retention time (min); KI (lit.): Kovátz index from the literature (Adams, 2007); KI (exp.): experimental Kovátz index; RA: relative area calculated from the peak area relative to the total peak area; SI: similarity index.

### Preliminary stability

As shown in Table II, all formulations tested in two stages of analysis were stable regarding organoleptic characteristics of color, odor, uniformity, brightness and absence of precipitates compared with the commercial reference product.

Concerning the pH determination, our results showed no significant differences between times  $t_0$  and  $t_{12}$  for the three samples. It indicates that neither acidic nor

alkaline decomposition products were generated during the preliminary stability test.

We also found that samples were stable at  $t_0$  and  $t_{12}$  in rotation, with no precipitates, coalescence and/or phase separation following a stability test under mechanical stress by centrifugation. These results show that samples exhibit physical stability even after increasing the force of gravity and mobility of the droplets.

**TABLE II** - Preliminary stability studies of formulations after heat stress

Parameters	Samples					
	IBCO5		IBCO10		Commercial	
	$t_0$	$t_{12}$	$t_0$	$t_{12}$	$t_0$	$t_{12}$
<b>Color</b>	W/OP	W/OP	W/OP	W/OP	W/OP	W/OP
<b>Odor</b>	C	C	C	C	C	C
<b>Uniformity</b>	C	C	C	C	C	C
<b>Brightness</b>	C	C	C	C	C	C

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**TABLE II** - Preliminary stability studies of formulations after heat stress

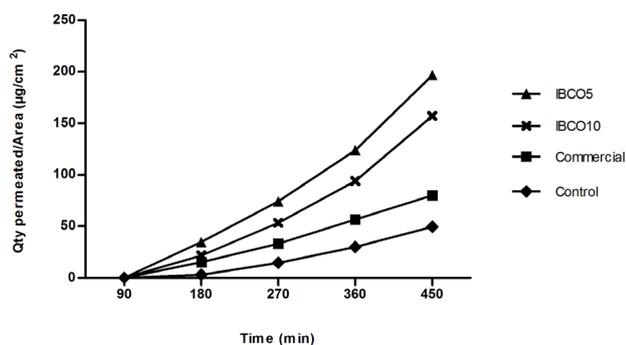
Parameters	Samples					
	IBCO5		IBCO10		Commercial	
	t <sub>0</sub>	t <sub>12</sub>	t <sub>0</sub>	t <sub>12</sub>	t <sub>0</sub>	t <sub>12</sub>
Absence of precipitates	C	C	C	C	C	C
pH	3.71±0.03	3.74±0.02	3.86±0.02	3.97±0.03	4.55±0.01	4.61±0.02
Stability against mechanical stress	C	C	C	C	C	C
IBU content (%)	100.5±0.04	95.1±0.02	101±0.06	98.4±0.03	100.4±0.02	99.1±0.04

W/OP: white and opaque; C: conform; t<sub>0</sub>: 24 hours after preparation, t<sub>12</sub>: 12 days after preparation (n=3).

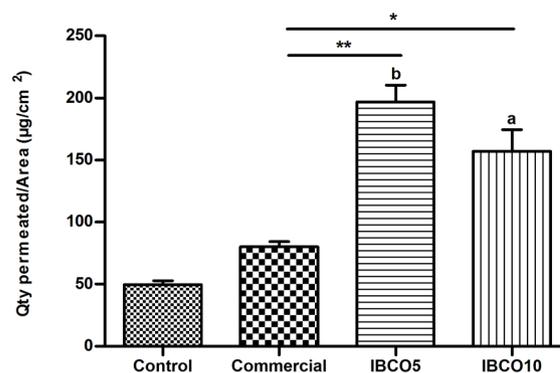
### Ex vivo permeation and penetration study

The drug permeation through pig ear skin, between donor and receiver compartment, in vertical diffusion cells, were tested for all the samples. The highest amounts of IBU permeated after 450 minutes were observed from those samples containing copaiba oil, as shown on Figures 1 and 2.

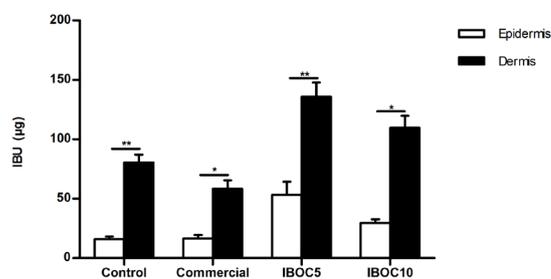
When we evaluate the ability of copaiba oil as an IBU permeation enhancer through the pig ear skin membrane, was observed a higher distribution of this drug in dermis when compared to epidermis (Figure 3).



**FIGURE 1** - Release of ibuprofen (IBU) over time - cumulative permeation curves of IBU from cream formulations containing 5 or 10% of copaiba oil in comparison to a control and commercial product. It represents the average of total IBU amount transferred by a diffusion area using pig ear skin as the membrane model monitored for 450 minutes (n=3).



**FIGURE 2** - Cutaneous permeation of ibuprofen (IBU) through pig skin at 450 min in vertical diffusion cells. \*p < 0.05; \*\*p < 0.01 in comparison with Commercial product; <sup>a</sup>p < 0.05 and <sup>b</sup>p < 0.01 in comparison with control (CO) (n=3).



**FIGURE 3** - Skin distribution of ibuprofen (IBU) in the epidermis and the dermis, 450 minutes application of cream with IBU 5% (CO), Commercial product, cream with IBU 5% and copaiba oil 5% (IBCO5) and cream with IBU 5% and copaiba oil 10% (IBCO10). \*p < 0.05; \*\*p < 0.01 in comparison with epidermis and dermis (n = 3).

The effects of copaiba oil on IBU release through permeation at 450 min were further substantiated by

permeation flux ( $J$ ,  $\mu\text{g cm}^{-2} \text{h}^{-1}$ ) of IBU through pig ear skin (Table III).

**TABLE III** - Steady-state flux, permeability coefficient and lag time of the samples

Samples	$J$ ( $\mu\text{g cm}^{-2} \text{h}^{-1}$ )	$K_p$ ( $\text{cm h}^{-1}$ )*	$T_L$ (min.)
Control	$10.32 \pm 1.52$	1.03	120
Commercial	$14.44 \pm 2.39$	1.44	90
IBCO5	$35.72 \pm 6.35$	3.57	90
IBCO10	$29.78 \pm 2.41$	2.98	90

$J$ : Steady-state flux;  $K_p$ : Permeability coefficient;  $T_L$ : Lag time.

\*Three cells analyzed expressed as mean  $\pm$  SEM.  $n=3$

Based on the latency period, it was possible to define the kinetics of the permeation of three mathematical models (Higuchi, first order or zero

order), aiming to determine the order of the process. The linear correlation coefficient ( $R^2$ ) for each model was determined (Table IV).

**TABLE IV** - Cutaneous permeation kinetic patterns of IBU and their linear correlation coefficients ( $n=3$ )

Mathematical models	Linear correlation coefficients ( $R^2$ )			
	Control	Commercial	IBCO5	IBCO10
Higuchi	0.8773	0.9592	0.9322	0.9054
First order	0.9412	0.9913	0.9749	0.9585
Zero order	0.9415	0.9915	0.9759	0.9594

## DISCUSSION

Several studies reported the use of the GC-MS technique for qualitative and quantitative analysis of copaiba oil. This chromatographic technique was effective in this study since all identified terpenes by GC-MS in copaiba oil were known from other copaiba oil samples and match those already reported in the literature (Dias, *et al.*, 2014; Custodio, Veiga-Junior, 2012).  $\beta$ -caryophyllene was the main detected compound, which is in agreement with previous chromatographic studies that have shown  $\beta$ -caryophyllene as the major compound found in copaiba oil samples (Leandro *et al.*, 2012).

Stability studies performed to evaluate IBU content in IBCO5, IBCO10 and commercial product, at  $t_0$  and  $t_{12}$  after heat/cool stress, are in accordance with the specification range set by the British Pharmacopoeia, 2014 (95 to 105%). These data demonstrated that even after a period of heat/cool stress, samples were able to maintain their chemical stability, showing that the formulations meet the pharmacopeial specification.

Based on the quantification of IBU obtained from HPLC, we observed drug permeation through pig ear skin between donor and receiver compartment in vertical diffusion cells. This pattern was seen in all samples tested (Figure 1). It has been previously shown that several

commercial formulations containing IBU 5% in gel increased drug permeation through porcine skin for 8 h (Herkenne *et al.*, 2007).

IBCO5 and IBCO10 samples only differ from others by having the copaiba oil in their compositions. The highest amounts of IBU permeated after 450 minutes were observed on those samples containing that oil. It thus indicates that since the copaiba oil contains a significant terpene composition which may play an important role in promoting cutaneous permeation of IBU through porcine skin (Figure 1). Interestingly, some studies found that the permeation enhancement capacities of the crude natural oils were significantly higher than its main terpene components (Lan *et al.*, 2014; Monti *et al.*, 2002).

Cutaneous permeation of IBU through pig skin shows a remarkable release of the drug at 450 min in the presence of copaiba oil (IBCO5 and IBCO10) (Figure 1). Both IBCO5 and IBCO10 showed significant differences ( $p < 0.01$  and  $p < 0.05$ , respectively) when compared with control or commercial product (Figure 2). Interestingly, IBCO5 induced a two-fold increase in IBU release in comparison with the commercial product. On the other hand, higher amount of copaiba oil (10%) (IBCO10) did not induce further release of IBU than the formulation with 5% (IBCO5). In addition, the commercial product showed no difference in comparison with control (Figure 2). Therefore, despite the fact that the copaiba oil demonstrates permeation enhancement activity of IBU, our data suggests that copaiba oil is not more efficient when its concentration is doubled.

IBCO5 and IBCO10 showed significantly higher steady-state flux ( $J$ ) of IBU through pig ear skin ( $p < 0.01$  and  $p < 0.05$ , respectively) than control or commercial product. Moreover, copaiba oil at 10% (IBCO10) did not increase permeation flux when compared with a lower concentration (Table III). A similar result was found by Oliveira *et al.* (2010) when they compared the skin permeation efficacy of kojic acid using two different concentrations of copaiba oil (25% and 50%) in vertical Franz diffusion cells. There wasn't a statistically significant difference between these two concentrations when the steady-state flux was evaluated. This study concluded that both concentrations could be used as skin permeation enhancers for hydrophilic drugs.

Analyzing the lag or latency time, which is the time that the drug takes to start its permeation, a shorter time (90 min) was found for IBCO5, IBCO10 and the commercial product than the control (120 min). These differences in lag time can be explained by the absence of copaiba oil as skin permeation enhancer in the control, and copaiba oil was used for IBCO5 and IBCO10 and the commercial product contains nerolidol, an amphiphilic sesquiterpene also considered a drug penetration enhancer due its capacity for the disruption of the highly organized lipid packing in the stratum corneum (Chen *et al.*, 2016).

According to our results, all samples followed a zero order model as a permeation kinetic profile due to their higher coefficient. This model indicates that the steady-state flux ( $J$ ) is constant for the interval of calculated time (after lag time) and independent of the drug concentration used. This kinetic model is typical for formulations with infinite doses (Sato *et al.*, 2007; Ruela *et al.*, 2016).

Following permeation experiments, the amount of IBU was determined in different skin layers after their separation as described in Section 2.5. Very significant differences were observed in all formulations after 450 min, including IBCO5 ( $p < 0.01$ ) and IBCO10 ( $p < 0.05$ ). Importantly, at least a two-fold amount of IBU was found in the porcine dermis in comparison with epidermis (Figure 3). The amount of IBU accordingly increased with the depth of the skin at a certain time mainly in the presence of copaiba oil. This finding supports the hypothesis of a unique role played by copaiba oil as a skin permeation enhancer of IBU.

## CONCLUSION

Our physical and chemical studies indicate the feasibility of developing stable IBU formulation with copaiba oil as permeation enhancer. In addition, permeation and penetration data suggests that copaiba oil at 5% and 10% incorporated into an emulsified base not only markedly improved drug skin permeation but also showed penetration enhancing properties of IBU in the dermis. As a result, copaiba oil increased the depth of IBU penetration in skin layers. Altogether, our study demonstrates that copaiba oil is an attractive tool to be explored as a potent cutaneous enhancer of drugs such as IBU.

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## REFERENCES

- Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry. Allured Publishing Corporation: Carol Stream; 2007. 457p.
- Bello AI, Kuwornu S. 5% Ibuprofen iontophoresis compared with transcutaneous electrical nerve stimulation in the management of knee osteoarthritis: a feasibility study. *Op J Ther Rehab*. 2014;2(4):166-172.
- British Pharmacopoeia, The Stationery Office: London; 2014.
- Chen J, Jiang Q, Chai Y, Zhang H, Peng P, Yang X. Natural terpenes as penetration enhancers for transdermal drug delivery. *Molecules*. 2016;21(12):1709.
- Custodio DL, Veiga-Junior VF. True and common balsams. *Rev Bras Farmacogn*. 2012;22(6):1372-1383.
- Dias DS, Fontes LBA, Crotti AEM, Aarestrup BJV, Aarestrup FM, Da Silva Filho AA, Corrêa JOA. Copaiba oil suppresses inflammatory cytokines in splenocytes of C57Bl/6 mice induced with Experimental Autoimmune Encephalomyelitis (EAE). *Molecules*. 2014;19(8):12814-12826.
- Herkenne C, Naik A, Kalia YN, Hadgraft J, Guy RH. Ibuprofen transport into and through skin from topical formulations: *In vitro* – *In vivo* comparison. *J Invest Dermatol*. 2007;127(1):135-142.
- Herman A, Herman AP. Essential oils and their constituents as skin penetration enhancer for transdermal drug delivery: a review. *J Pharm Pharmacol*. 2015;67(4):473-485.
- Hussain A, Khan GM, Shah SU, Shah KU, Rahim N, Wahab A, Rehman AU. Development of a novel ketoprofen transdermal patch: Effect of almond oil as penetration enhancers on *in vitro* and *ex vivo* penetration of ketoprofen through rabbit skin. *Pak J Pharmac Sci*. 2012;25(1):227-232.
- Jiang Q, Wu Y, Zhang H, Liu P, Yao J, Yao P, et al. Development of essential oils as skin permeation enhancers: penetration enhancement effect and mechanism of action. *Pharm Biol*. 2017;55(1):1592-1600.
- Khan NR, Khan GM, Wahab A, Khan, AR, Hussain A, Nawaz A, et al. Formulation, and physical, *in vitro* and *ex vivo* evaluation of transdermal ibuprofen hydrogels containing turpentine oil as penetration enhancer. *Pharmazie*. 2011;66(11):849-852.
- Lan Y, Li H, Chen Y, Zhang Y, Liu N, Zhang Q, et al. Essential oil from *Zanthoxylum bungeanum* Maxim. and its main components used as transdermal penetration enhancers: a comparative study. *J Zhejiang Univ Sci B*. 2014;15(11):940-52.
- Leandro L. Vargas FS, Barbosa PCS, Neves JKO, Silva JA, Veiga-Junior VF. Chemistry and biological activities of terpenoids from copaiba (*Copaifera spp.*) Oleoresins. *Molecules*. 2012;17(4):3866-3889.
- Lucca LG, Matos SP, Borille BT, Dias DO, Teixeira HF, Veiga-Junior VF, et al. Determination of  $\beta$ -caryophyllene skin permeation/retention from crude copaiba oil (*Copaifera multijuga* Hayne) and respective oil-based nanoemulsion using a novel HS-GC/MS method. *J Pharm Biolmed Anal*. 2015;104:144-148.
- McLafferty FW. Wiley Registry of Mass Spectral Data, 8<sup>th</sup> ed., New York: Hoboken, John Wiley & Sons; 2008.
- Monti D, Chetoni P, Burgalassi S, Najarro M, Saettone MF, Boldrini E. Effect of different terpene-containing essential oils on permeation of estradiol through hairless mouse skin. *Int J Pharmac*. 2002;237(1-2):209-214.
- Obata Y, Ashitaka Y, Kikuchi S, Isowa K, Takayama K.. A statistical approach to the development of a transdermal delivery system for ondansetron. *Int J Pharm*. 2010;399(1-2):87-93.
- Oliveira RVM, Ohara MT, Vila MMDC, Gonçalves MM. *In vitro* evaluation of copaiba oil as a kojic acid skin enhancer. *Braz J Pharm Sci*. 2010;46(2):363-370.
- Padula C, Nicoli S, Santi P. *In vitro* evaluation of bioadhesive films containing ibuprofen. *Anti-Inflam Anti-Aller Ag Medic Chem*. 2011;10(3):240-245.
- Pontrelli G, Monte F. A two-layer model for transdermal drug delivery and percutaneous absorption. *Math Biosci*. 2014;257:96-103.
- Ruela ALM, Perissinato AG, Lino MES, Mudrik PS, Pereira GR. Evaluation of skin absorption of drugs from topical and transdermal formulations. *Braz J Pharm Sci*. 2016;52(3):527-544
- Santis AK, Freitas ZMF, Ricci-Junior E, Brito-Gitirana L, Fonseca LB, Santos EP. Nifedipine in semi-solid formulations

for topical use in peripheral vascular disease: preparation, characterization, and permeation assay. *Drug Develop Ind Pharm.* 2012;39(7):1098-1106.

Sarkar AB, Dudley R, Melethil S, Speidel J, Markandakumar B. Chemical stability of hydrocortisone in topical preparation in proprietary VersaPro™ cream base. *Innov Pharm.* 2011;2(3):1-3.

Sato MEO, Gomara F, Pontarolo R, Andrezza IF, Zaroni M. Permeação cutânea *in vitro* do ácido kójico. *Braz J Pharmac Sci.* 2007;43(2):195-203.

Simon A, Amaro MI, Healy AM, Cabral LM, Sousa VP. Comparative evaluation of rivastigmine permeation from a transdermal system in the Franz cell using synthetic membranes and pig ear skin with *in vivo-in vitro* correlation. *Int J Pharm.* 2016;512(1):234-241.

Stahl J, Wohlert M, Kietzmann M. The effect of formulation vehicles on the *in vitro* percutaneous permeation of ibuprofen. *BMC Pharmacol.* 2011;11(12):1-5.

Veiga-Junior VF, Rosas EC, Carvalho MV, Henriques MGMO, Pinto AC. Chemical composition and anti-inflammatory activity of copaiba oils from *Copaifera cearensis* Huber ex Ducke, *Copaifera reticulate* Ducke and *Copaifera multijuga* Hayne - A comparative study. *J Ethnopharmacol.* 2007;112(2):248-254.

Yuan X, Capomacchia AC. The binary eutectic of NSAIDs and two-phase liquid system for enhanced membrane permeation. *Pharmac Develop Techn.* 2005;10(1):1-10.

Zamarioli CM, Martins RM, Carvalho EC, Freitas LAP. Nanoparticles containing curcuminoids (*Curcuma longa*): development of topical delivery formulation. *Rev Bras Farmacogn.* 2015;25(1):53-60.

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