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Evaluation of the transdermal permeation of different paraben combinations through a pig ear skin model

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ABSTRACT

Although parabens have several features of ideal preservatives, different studies have shown that they may affect human health due to their estrogenic activity. Therefore, various strategies have been applied to reduce their skin penetration. However, the effect of paraben combinations on transdermal permeation has not yet been investigated. Thus, the objective of this study was to evaluate paraben permeation in pig ear skin using a Franz diffusion cell system with capillary electrophoresis detection, in order to identify which paraben combinations (defined by a factorial design) have the lowest skin permeation. The permeation of isolated parabens was also evaluated and the permeation characteristics, obtained by the Moser model, confirmed that lipophilicity and molecular weight may influence the systemic absorption of these compounds. In previous tests using isolated parabens, methyl and ethyl parabens presented greater retention in the epidermis compared to the dermis, while propyl and butyl parabens had similar retention profiles in these layers. An increase in ethanol concentration and experimental time promoted greater parabens retention in the dermis compared to the epidermis. The binary combinations of methyl and ethyl parabens as well as of methyl and propyl parabens (added to several cosmetic products in order to increase the antimicrobial spectrum) reduced significantly their permeation rates through pig ear skin (with the exception of EP), probably due to the high retention of these parabens in the epidermis and dermis.

1. Introduction

Parabens are derivatives of hydroxybenzoic acid esterified at the C-4 position and are widely used as preservatives in food, cosmetics and pharmaceutical products. The most common parabens used in cosmetic products are methyl (MP), ethyl (EP), propyl (PP) and butyl (BP). Their preservative activity increases with length of the alkyl group, from methyl to n-butyl (Pedersen et al., 2007), and they show inhibitory effects on microbial membrane transport and mitochondrial function (Soni et al., 2005).

Cosmetic can contain several of these compounds or just one of them (El Hussein et al., 2007). Some combinations of parabens may reduce their efficacy, while other combinations have shown synergistic effects and provide preservation against a broad range of microorganisms. For instance, the combination of MP and PP is often added to topical aqueous formulations due to their antimicrobial synergistic effects (Soni et al., 2005). This same association, in ethanolic solution, also provides an efficient antifungal action (Neves et al., 2009). In order to reach the best protection and minimize risks to the users, the employed paraben combinations should be ideally tailored according to the physicochemical properties of each formulation (Charnock and Finsrud, 2007). In addition, parabens can be combined with other types of preservatives to improve the antimicrobial efficacy (FDA, 2007).

Parabens posses several features of ideal preservatives, including a broad spectrum of antimicrobial activity, chemical stability in relation to pH (effective at pH 4.5–7.5) and temperature, as well as low cost (Maddox, 1982). On the other hand, they have exhibited estrogenic effects in animal models (Prusakiewicz et al., 2007). *Ex vivo* experimentation showed that MP potentiates UV-induced damage of skin keratinocytes (Handa et al., 2006). BP (Oishi, 2001) and PP (Oishi, 2002) also adversely affected testosterone synthesis and male reproductive functions in a rodent model. Most of these effects can be associated with the systemic absorption of parabens, due to their relatively high lipophilicity and low molecular weight, reinforcing the importance of studies that evaluate the risks of

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formulations containing parabens in terms of dermal absorption (Akomeah et al., 2007; El Hussein et al., 2007; Pedersen et al., 2007; Nicoli et al., 2008a,b).

Since the effect of paraben combinations on their transdermal permeation has not yet been studied, this study evaluated such effects in pig ear skin using a Franz diffusion cell system with capillary electrophoresis (CE) detection. Using a factorial design, the paraben combinations with the lowest skin permeation were identified. The secondary goals of this study included the development and validation of a CE quantification method, evaluation of the effect of partitioning characteristics (KH) and the diffusive parameter (D/H^2) on paraben permeation using the Moser et al. (2001) model, and determination of the retention of the parabens in the dermis and epidermis.

2. Materials and methods

2.1. Chemicals

MP, EP, PP and BP were purchased from Pharma Service Bioextract (São Paulo, BR). All chemicals used in the CE analysis were of analytical grade.

2.2. Permeation studies

Full thickness of pig ear skin (1.00 ± 0.05 mm) was obtained from young animals sacrificed at a local slaughterhouse (Antonio Carlos, SC, BR). The skin was initially cleaned with tap water, and then hairs and subcutaneous fat tissue were removed. The membranes obtained were stored at −80 °C for no longer than two months before use. Before the experiments, the skin was allowed to thaw at room temperature. The skin was mounted in a two-chamber glass Franz diffusion cell with the stratum corneum towards the donor chamber witch had an available diffusion area of 1.77 cm^2. The receiver chambers were filled with 10 ml of ethanol–phosphate buffer (EtOH/PBS, pH 7.2) to maintain the sink conditions (20% (v/v) when the parabens were tested separately—phase 1 and 50% (v/v) during the experiments with combined and isolated parabens—phase 2). As permeation studies with formulations containing single parabens were carried out with a different concentration of ethanol from the permeation studies of formulations with combined parabens, these results were not compared. Since ethanol has been recognized as a potential transdermal permeation enhancer, each of the four parabens was evaluated for permeation in both concentrations of ethanol (with only one repetition in phase 2). The system was kept at 37 °C by circulating heated water through an external water jacket, and the solution in the receiver chambers was continuously stirred at 900 rpm using Teflon coated magnetic stirrers. After 30 min, 2 ml of the paraben solution (0.1% paraben dissolved in an ethanol/PBS mixture—20/80 and 50/50 for phases 1 and 2, respectively) was introduced into the donor chamber, and the diffusion cells were covered with aluminum foil to prevent evaporation.

At fixed time intervals (total time of phase 1 = 6 h; phase 2 = 7.5 h), samples (400 μl) were withdrawn from the receiver chambers, replaced with the same volume of fresh medium and, subsequently, assayed by CE as described below. Experiments were repeated four times, except the factorials. At the end of the permeation experiment, skins were carefully washed with PBS in order to remove residual of donor solution. Samples of the epidermis were separated from the dermis using a scalpel and placed in separate pre-weighed tubes to determine the amount of each tissue sample collected. Parabens were extracted from the samples with 5 ml of methanol, centrifuged (22,000 × *g*; 10 min), sonicated (15 min), and filtered through cellulose membranes (0.45 μm; Millipore). The extraction method was validated in blank experiments and by spiking the skin with a known amount of each paraben. The permeated and retained amounts of parabens were determined using a previously validated CE method (see Section 2.4).

Regarding the permeation parameters, the steady-state permeation flux (J_s) was determined from the linear slope of the cumulative amount of paraben permeated vs. time curve. The lag time (T_L) represented the time required to achieve the steady-state flux, and the permeability coefficient (P) was the relation between the flux and the initial concentration of each paraben added to the donor compartment ($P = J_s/C_d$).

The permeability coefficients were also calculated according to the Moser et al. (2001) model, which does not assume the achievement of steady-state conditions and considers Fickĭs law. The partitioning characteristics (KH) and diffusive parameter (D/H^2) of the parabens were estimated.

2.3. Experimental design

A 2^3 factorial design was employed, including a triplicate of central point to estimate the experimental error (Armstrong and James, 1990; Montgomery, 2005). When combinations of parabens were used in the donor chamber, one paraben was maintained at a fixed concentration level and the other three were modified at two levels – low (−1) and high (+1) – where the low level implies the absence of dependent variables and the high level indicates their presence (1000 μg ml^{-1}). The factorial results were expressed as paraben flux values.

The instrumental variables (voltage, injection mode, detection wavelength, cartridge temperature and capillary dimensions) were kept constant during the experiments to reduce interference from the experimental error. The experiments were randomly performed to avoid distortion of the statistical results.

Multiple regression analysis was used to estimate the Beta regression model parameters and to evaluate the influence of factor level on the permeation flux, as well as the occurrence of synergism or antagonism among the parabens.

2.4. Paraben analysis

Paraben analysis were performed by CE (Hewlett-Packard Model ^{3D}CE, Agilent Technologies) using an instrument equipped with a diode-array detector, with automatic injector and sampler, using an uncoated fused-silica capillary of 40 cm (effective length 48.5 cm; 50 μm I.D.) with a normal light path. The experimental conditions included 30 mM of borate buffer as the background electrolyte (BGE), temperature of 25 °C, and voltage of 30 kV. Samples were injected hydrodynamically by applying 50 mbar pressure to the outlet vial for 10 s and detection was performed at 297 nm. New capillaries were conditioned by rinsing them with NaOH 1 mol L^{-1} for 1 h, deionized water for 20 min, and separation buffer for 10 min. Prior to each run, the capillary was rinsed with deionized water and BGE to obtain the best reproducibility. The data was processed and analyzed using Class VP software (Shimadzu, Kyoto, Japan). The sample pretreatment approach, developed to clean and concentrate the biological samples, was based on acetonitrile deproteinization (liquid–liquid extraction). 3,5-dinitrobenzoic acid was used as the internal standard (20 μg ml^{-1}).

The quantification method was validated according to ICH (1997) Guidelines. Calibration curves (1–40 μg ml^{-1}) were obtained using the least-squares regression method. The relative standard deviation values were calculated for repeated standard solutions sampling at different concentration levels (4, 15 and 32 μg ml^{-1}) to check the method repeatability. To determine the intermediate precision, the same experiments were performed on six different days. The developed method was linear ($R^2 > 0.9999$),

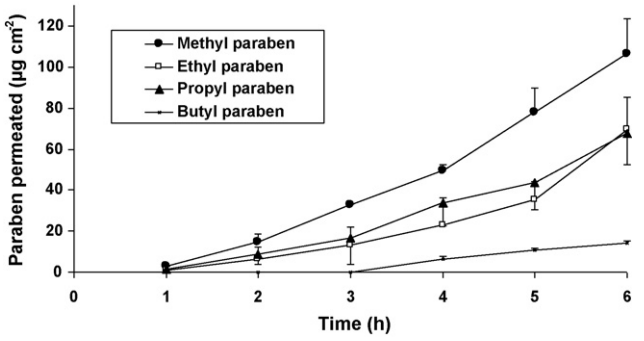

Fig. 1. Permeation profiles of methyl, ethyl, propyl and butyl paraben in pig ear skin. Results are presented as mean ± SDM ($n = 4$) and the statistical analysis was conducted separately for each paraben.

precise (intraday and interday relative standard deviations <2.97 and 3.22, respectively), accurate (ranged from 98 to 110%) and specific. Therefore, this method allowed the MP, EP, PP and BP quantification, despite the presence of interfering proteins from the pig ear skin.

2.5. Statistical analysis

The results were expressed as mean ± standard deviation and statistical differences were determined using one-way ANOVA and the Student–Newman–Keuls (SKN) test for multiple comparisons, with a significance level of $p<0.05$.

3. Results and discussion

3.1. Permeation and retention of isolated parabens (phase 1)

The *ex vivo* transdermal permeation of isolated parabens was initially studied, followed by the evaluation of paraben combinations using a factorial design. When the permeation profiles (paraben permeated vs. time) of isolated parabens were compared, it could be seen that the values increased with the size of the alkyl chain. Thus, MP presented higher permeation, followed by EP and PP, while BP had the lowest permeation (Fig. 1). The diverse solubility and lipophilicity of the molecules could account for the different permeation profiles.

The permeation parameters obtained in this study are shown in Table 1. Even though a small increase in the lag time values was measured for MP when compared to EP (approximately 8.6%), and between EP and PP (approximately 7.5%), the differences were statistically significant only when the lag time of BP was compared to the other parabens (96.4, 80.9 and 68.4% for MP, EP and PP, respectively). Pedersen et al. (2007) showed a rapid permeation of EP through rabbit ear skin with a lag time of around zero. Akomeah et al. (2007) evaluated MP and BP permeation through human epidermis and the lag time values were 0.288 ± 0.093 and 0.450 ± 0.109 h, respectively. The different tissues and animal species used could account for the discrepancies between these data. Akomeah et al. (2007) used only human epidermis, whereas this study considered pig dermis and epidermis, increasing the diffusion barrier, which could be the reason for the higher lag time values obtained by this study.

Table 2
Permeation parameters of parabens through pig ear skin according to Moser model.

Paraben	KH (cm)	D/H^2 (cm^{-1})	P ($cm\,h^{-1}$)
Methyl paraben	0.207 ± 0.097	0.111 ± 0.032	0.02148 ± 0.004
Ethyl paraben	0.350 ± 0.090	0.059 ± 0.018	0.01975 ± 0.001
Propyl paraben	0.156 ± 0.061	0.073 ± 0.038	0.01019 ± 0.0015
Butyl paraben	0.066 ± 0.072	0.045 ± 0.001	0.00313 ± 0.00016

Mean ± standard deviation ($n = 4$); KH = partitioning characteristics; D/H^2 = diffusive parameter; P = permeability coefficient.

When the permeation flux values of the parabens evaluated in this study were compared, the influence of the lipophilicity of the molecules was noted, since the reduction in permeation flux followed the order of increasing lipophilicity (Table 1). MP presented the highest permeation flux, followed by EP, PP and BP ($p < 0.05$, SNK; except PP vs. BP). The same results were obtained when the permeability coefficients were compared, since the initial concentration of each paraben was fixed (0.1%, w/v).

The MP and BP flux values were lower than those obtained by Akomeah et al. (2007), which could be the result of the use of different membranes. However, similar permeability coefficients were obtained for MP ($P = 0.021$ cm/h—literature vs. $p = 0.021$ cm/h—experiment), which could be due to the fact that the epidermis represents, in this case, a rate-limiting barrier. A greater difference was observed for BP ($P = 0.010$ cm/h—literature vs. $p = 0.002$ cm/h—this experiment). In this case, since dermis is more hydrophilic, this layer seems to be more important than the epidermis when it comes to the transdermal permeation, which could limit the permeation of higher lipophilic compounds such as BP.

The permeation parameters obtained according to the Moser et al. (2001) model are shown in Table 2. MP and PP had similar KH values to those obtained by Nicoli et al. (2008a). BP had the lowest KH value, which may be due to its longer lag time (2.3 h), or its low permeability coefficient. The statistical extrapolation of the permeated amounts of each paraben at different time intervals, after 6 h, indicated that the partitioning and the permeability characteristics were greater for BP (data not shown). This suggests that BP interacts with pig ear skin to a greater extent than with rabbit ear skin (Nicoli et al., 2008a).

The lowest diffusive parameter (D/H^2) was observed for BP, and the highest for MP (approximately 1.53 times higher than PP and 1.89 times higher than EP). The diffusion of parabens through skin depends on their interaction with intercellular lipids and their molecular weight. Generally, a strong interaction with lipids produces a decrease in diffusion, and this interaction increases with the lipophilicity.

The MP and EP permeability coefficients (KH^*D/H^2) were not statistically different ($p > 0.05$) and were higher than those of PP and BP.

After the permeation experiments (6 h), the final amount of each paraben retained in the epidermis and dermis revealed significant differences between these layers, for each paraben individually (Fig. 2). MP and EP showed greater retention in the epidermis

Table 1
Permeation parameters of isolated parabens.

Parameter	Methyl paraben	Ethyl paraben	Propyl paraben	Butyl paraben
J_S (mean ± sd) ($\mu g/cm^2\,h$)	$20.657 \pm 4.133^{(a)}$	$14.288 \pm 2.108^{(b)}$	$7.274 \pm 3.205^{(c)}$	$2.199 \pm 0.074^{(c)}$
T_L (mean ± sd) (h)	$1.168 \pm 0.281^{(a)}$	$1.267 \pm 0.190^{(a)}$	$1.362 \pm 0.388^{(a)}$	$2.294 \pm 0.208^{(b)}$
P (mean ± sd) (cm/h)	$0.021 \pm 0.004^{(a)}$	$0.014 \pm 0.002^{(b)}$	$0.007 \pm 0.003^{(c)}$	$0.002 \pm 7.439 \times 10^{-5(c)}$

ANOVA/SNK tests ($p < 0.05$) were carried out as appropriate ($n = 4$). Different letters indicate significantly statistical differences among treatments. Each permeability parameter was analyzed separately.

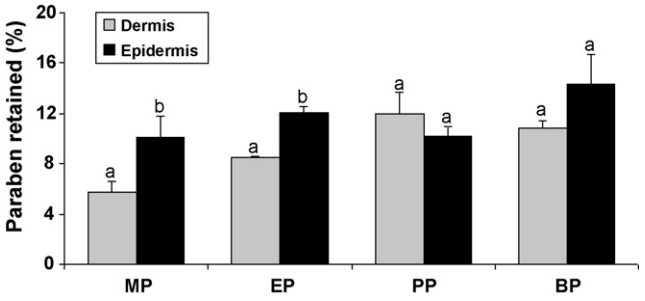

Fig. 2. Percentages of methyl (MP), ethyl (EP), propyl (PP) and butyl (BP) paraben retained in the dermis and epidermis after 6 h. Mean ± SDM ($n = 4$). ANOVA/SNK tests ($p < 0.05$) were carried out as appropriate. Different letters indicate significantly statistical differences between treatments.

compared to the dermis, while PP and BP presented similar retention profiles in these layers ($p > 0.05$). The differential parabens lipophilicity and the different lipid contents of the skin layers could be the reason for these retention differences. The presence of stratum corneum in the epidermis increases its lipophilicity, a fact that could explain the higher paraben amount retained in this layer.

In summary, the similarity of these results with data from the literature (El Hussein et al., 2007; Pedersen et al., 2007; Nicoli et al., 2008a) confirmed that lipophilicity influences the permeation level, i.e., the most lipophilic parabens (BP > PP > EP > MP) take longer to cross the skin barrier, and have lower flux values (BP = PP < EP < MP).

3.2. Permeation of the paraben combinations (phase 2)

In this second experimental phase, a solution of 50% ethanol–phosphate buffer (v/v) was used to enhance the permeation of the combined parabens. An increase in the amount of permeated paraben was observed and also a larger difference among the treatments, which allowed calculation of the permeation parameters with greater reliability. In most cases, when a concentration of 20% ethanol–phosphate buffer (v/v) was used as the dissolution medium for the parabens, the dermis presented a lower retention in comparison to the epidermis. On the other hand, an increase in the ethanol concentration from 20 to 50% (v/v) in the dissolution medium, as well as a greater experimental time (7.5 h) during the second experimental phase, promoted a greater parabens accumulation in the dermis, probably due to greater permeation (Fig. 3).

Since parabens are used in combination, especially MP and PP, in order to enhance their antimicrobial effects (Soni et al., 2005), evaluation of the effect of paraben interactions on their transdermal permeation is very relevant.

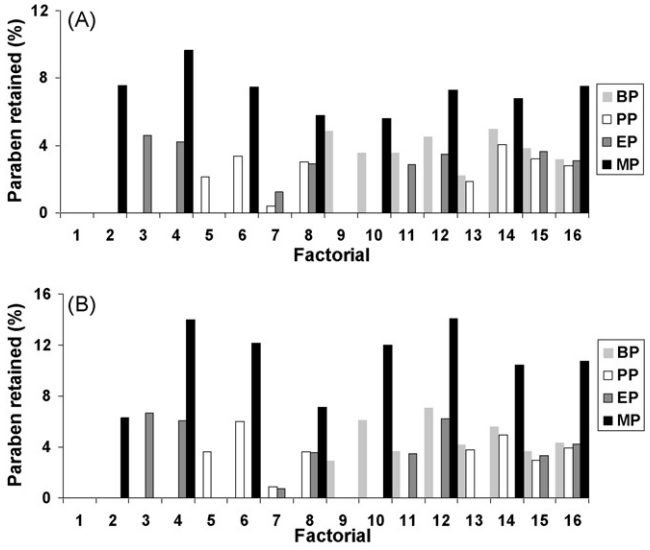

Fig. 3. Percentages of methyl (MP), ethyl (EP), propyl (PP) and butyl (BP) paraben retained in the epidermis (A) and dermis (B) after 7.5 h when different experimental units (factorials) were evaluated. Factorials 2, 3, 5 and 9 represent the amounts of isolated parabens retained in these skin layers, factorial 1 a control without paraben, and other factorials the binary, tertiary and quaternary combinations of these compounds.

The flux values obtained for triplicate central points were very different, which could be attributed to intra-species and experimental variations. A qualitative analysis of these results showed that binary combinations of PP with other parabens had reduced flux values (approximately 2 times for MP and BP, and 8.3 times for EP) compared with the isolated parabens. These results may be associated with the physicochemical properties, since the solubility of PP decreases by 50% in relation to its solubility in pure water when EP is present, whereas the respective solubilities of the other parabens only change within a range of 10% (Giordano et al., 1997). This difference in solubility could change the partitioning characteristics of these molecules within the skin layers, which would explain the different permeation flux values of binary combinations in relation to those of the isolated parabens. Furthermore, the analysis of retained paraben in the dermis and epidermis revealed the lowest retention for the binary combination of EP and PP in relation to the other combinations (around 3.9 and 9.2 times for EP and 5.2 and 4 times for PP, in the epidermis and dermis, respectively; Fig. 3), indicating that an interaction between these parabens may occur in the acceptor phase. The combinations of three parabens reduced to approximately half the flux values, except for the combination MP–PP–BP, since BP had a greater flux, which was not observed when it was tested individually. When

Table 3
The 2^3 factorial design matrix and obtained responses [J_S = flux ($\mu g\,cm^{-2}$)] on permeation studies of paraben combinations through pig ear skin.

Experiment	*A*	*B*	*C*	Responses factorial 1 (R_{MP})	Responses factorial 2 (R_{EP})	Responses factorial 3 (R_{PP})	Responses factorial 4 (R_{BP})
1	−1	−1	−1	19.2	15.2	15.1	16.0
2	1	1	−1	16.3	15.4	18.1	16.2
3	−1	1	−1	19.1	11.0	12.2	15.0
4	1	1	−1	18.9	17.0	18.0	16.3
5	−1	−1	1	17.7	14.7	16.1	16.9
6	1	−1	1	18.8	16.3	17.1	17.1
7	−1	1	1	16.5	16.2	16.3	16.9
8	1	1	1	21.0	18.3	18.3	18.7
9	0	0	0	19.9	16.5	16.6	15.9
10	0	0	0	18.9	16.8	16.8	17.0
11	0	0	0	18.0	15.3	15.1	15.6

Factorial 1: A (EP), B (PP) and C (BP); *factorial 2*: A (MP), B (PP) and C (BP); *factorial 3*: A (MP), B (EP) and C (BP); *factorial 4*: A (MP), B (EP) and C (PP); *levels* ($mg\,L^{-1}$): (−) 0.000; (0) 500.0; (+) 1000; R_{MP}, R_{EP}, R_{PP} and R_{BP} represent normalized responses (angular coefficient).

Table 4
Statistical treatment of permeation flux values obtained for paraben combinations according to the 2^3 factorial design.

Factorial 1	[a]Effect$_{MP}$	Factorial 2	[b]Effect$_{EP}$	Factorial 3	[c]Effect$_{PP}$	Factorial 4	[d]Effect$_{BP}$
Mean	18.58	Mean	15.70	Mean	16.34	Mean	16.50
EP	0.66	MP	2.49*	MP	2.95*	MP	0.86
PP	0.89	PP	0.25	EP	−0.38	EP	0.22
BP	0.14	BP	1.72	BP	1.13	PP	1.52
EP*PP	1.52	MP*PP	1.57	MP*EP	0.96	MP*EP	0.68
EP*BP	2.14	MP*BP	−0.64	MP*BP	−1.46	MP*PP	0.15
PP*BP	−0.36	PP*BP	1.53	EP*BP	1.08	EP*PP	0.64
EP*PP*BP	0.19	MP*PP*BP	−1.30	MP*EP*BP	−0.46	MP*EP*PP	0.13

[a,b,c,d]Effect values higher than 2.91, 2.39, 2.88, 2.13 present significance for columns effect$_{MP}$, effect$_{EP}$, effect$_{PP}$, effect$_{BP}$, respectively.
* Significant effects for alpha of 0.05%.

all parabens were combined, a permeation flux reduction of each paraben was observed. However, the statistical evaluation of these results was not able to detect significant differences among the tested paraben combinations. Therefore, the data was normalized according to the final amount of each paraben permeated (after 7.5 h) and the effects obtained were compared.

The factorial design matrix and the responses obtained (flux values after normalization) are shown in Table 3. The level of one paraben was kept constant, while the concentrations of the other parabens were modified. For instance, in factorial 1, MP concentration was maintained at 1000 $\mu g\,ml^{-1}$, whereas EP, PP and BP concentrations ranged from 0 to 1000 $\mu g\,ml^{-1}$, according to the experimental design. The same analysis was used for factorials 2–4. The qualitative analysis (Table 3) showed that the permeation flux values fluctuated according to the permeation of the paraben combinations.

When the normalized data was analyzed (Table 4), binary combinations of MP with EP (factorial 2) and MP with PP (factorial 3) resulted in a significant reduction in the permeation flux values. For factorials 1 and 4, the effects were not statistically significant. For the binary combinations of MP with EP and MP with PP, an increase of parabens stored in the dermis and epidermis was detected, except for EP in which a small reduction was observed in relation to the isolated paraben (Fig. 3). Whereas an interaction of PP and EP occurs in acceptor phase when these parabens are combined due to the low permeation flux values and low retention in the skin, this hypothesis cannot be considered for binary combinations of MP with EP or MP with PP, since a high retention was observed in the dermis and epidermis. In these cases, a reduction in the systemic delivery rate of these parabens from the dermis could be shown, since low flux values were obtained for these two binary combinations.

Since these binary combinations decreased the permeation flux of the parabens, it could be possible that the addition of permeation retardants to these combinations would affect this parameter significantly. Additionally, the formulation types and their components also may affect the absorption and antimicrobial activity of the parabens, as demonstrated by Dal Pozzo and Pastori (1996). According to these authors, emulsions can strongly influence the concentration of antimicrobial agents in the aqueous phase (and consequently their preservative activity), as well as the absorption of parabens through skin. Since commercial preparations are often applied to large areas of skin, low penetration rates can result in considerable amounts of parabens entering the body. Hence, a low penetration rate (near zero) must be achieved to ensure greater safety for the topical use of these parabens containing products. Therefore, it is necessary to select an appropriate paraben combination as well as component formulations (including transdermal permeation retardants) in order to minimize the systemic absorption and maximize the preservative effect of the parabens. Combinations that reduce the total paraben concentration in the formulation without compromising the degree of antimicrobial protection, and which result in a minimal systemic absorption, are preferable for use in the manufacturing of cosmetic products.

4. Conclusions

The permeation parameters of isolated parabens confirmed the hypothesis that lipophilicity and molecular weight influence the transdermal permeation of parabens through pig ear skin. The binary combinations of MP with EP and MP with PP (present in several cosmetic products in order to increase the antimicrobial spectrum) significantly reduced the permeation flux values of these mixtures, probably due to the high retention of these parabens in the epidermis and dermis, except for EP. In these cases, it is possible that the addition of a permeation retardant (for instance, nicotinamide) will reduce this parameter significantly, thus, reducing the adverse effects associated with the systemic absorption of these preservatives. It is also necessary to evaluate the dermal absorption risks of those formulations containing parabens in association with penetration enhancers or retardants.

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References

Akomeah, F.K., Martin, G.P., Brown, M.B., 2007. Variability in human skin permeability *in vitro*: comparing penetrants with different physicochemical properties. J. Pharm. Sci. 96, 824–834.
Armstrong, N.A., James, K.C., 1990. Factorial design of experiments. In: Understanding Experimental Design and Interpretation in Pharmaceutics. Ellis Horwood, London, pp. 27–55.
Charnock, C., Finsrud, T., 2007. Combining esters of para-hydroxy benzoic acid (parabens) to achieve increased antimicrobial activity. J. Clin. Pharm. Ther. 32, 567–572.
Dal Pozzo, A., Pastori, N., 1996. Percutaneous absorption of parabens from cosmetic formulations. Int. J. Cosmet. Sci. 18, 57–66.
El Hussein, S., Muret, P., Berard, M., Makki, S., Humbert, P., 2007. Assessment of principal parabens used in cosmetics after their passage through human epidermis-dermis layers (*ex vivo* study). Exp. Dermatol. 16, 830–836.
FDA (Federal Drug and Food Administration), 2007. Parabens. Date Prepared: 24-03-06, Revised: 31-10-07. Available at http://www.cfsan.fda.gov/~dms/cospara.html.
Giordano, F., Bettini, R., Donini, C., Gazzaniga, A., Caira, M.R., Zhang, G.G., Grant, D.J., 1997. Physical properties of parabens and their mixtures: solubility in water, thermal behavior, and crystal structures. J. Pharm. Sci. 88, 1210–1216.
Handa, O., Kokura, S., Adachi, S., Takagi, T., Naito, Y., Tanigawa, T., Yoshida, N., Yoshikawa, T., 2006. Methylparaben potentiates UV-induced damage of skin keratinocytes. Toxicology 227, 62–72.
ICH (International Conference on Harmonisation), 1997. Validation of analytical procedures: text and methodology. Available at http://www.ich.org/cache/compo/363-272-1.html.
Maddox, D.N., 1982. The role of p-hydroxybenzoates in modern cosmetics. Cosmet. Toilet. 97, 85–88.
Montgomery, D.C., 2005. Design and Analysis of Experiments, sixth ed. Wiley, New York.

Moser, K., Kriwet, K., Froehlich, C., Kalia, Y.N., Guy, R.H., 2001. Supersaturation: enhancement of skin penetration and permeation of a lipophilic drug. Pharm. Res. 18, 1006–1111.

Neves, E.R., Schäfer, S., Phillips, A., Canejo, J., Macedo, M.F., 2009. Antifungal effect of different methyl and propyl paraben mixtures on the treatment of paper biodeterioration. Int. Biodeteriorat. Biodegrad. 63, 267–272.

Nicoli, S., Padula, C., Aversa, V., Vietti, B., Wertz, P.W., Millet, A., Falson, F., Govoni, P., Santi, P., 2008a. Characterization of rabbit ear skin as a skin model for *in vitro* transdermal permeation experiments: histology, lipid composition and permeability. Skin Pharmacol. Physiol. 21, 218–226.

Nicoli, S., Zani, F., Bilzi, S., Bettini, R., Santi, P., 2008b. Association of nicotinamide with parabens: effect on solubility, partition and transdermal permeation. Eur. J. Pharm. Biopharm. 69, 613–621.

Oishi, S., 2001. Effects of butylparaben on the male reproductive system in rats. Toxicol. Ind. Health 17, 31–39.

Oishi, S., 2002. Effects of propyl paraben on the male reproductive system. Food Chem. Toxicol. 40, 1807–1813.

Pedersen, S., Marra, F., Nicoli, S., Santi, P., 2007. *In vitro* skin permeation and retention of parabens from cosmetic formulations. Int. J. Cosmet. Sci. 29, 361–367.

Prusakiewicz, J.J., Harville, H.M., Zhang, Y., Ackermann, C., Voorman, R.L., 2007. Parabens inhibit human skin estrogen sulfotransferase activity: possible link to paraben estrogenic effects. Toxicology 232, 248–256.

Soni, M.G., Carabin, I.G., Burdock, G.A., 2005. Safety assessment of esters of p-hydroxybenzoic acid (parabens). Food Chem. Toxicol. 43, 985–1015.