Comparative permeation study of caffeine and diclofenac gel formulations using artificial, human, and porcine skin barriers

Membranes tested		
Membrane Name	Abbreviation	Features of membrane
Permeaskin OLD	Permeaskin	
PermeaPad® Skin barrier Batch 1	Batch 1	
PermeaPad® Skin barrier Batch 1.2	Batch 1.2	Same day of Batch 1
PermeaPad® Skin barrier + filter nass	Batch filter nass	Same features of Batch 1, but changement of production process
PermeaPad® Skin barrier + Ceramides	Batch skin plus	More ceramides
PermeaPad® Skin barrier Batch 2	Batch 2	Higher lipid content compared to Batch 1
PermeaPad® Skin barrier Batch 2.1	Batch 2.1	Lower lipid content compared to Batch 2, but higher than Batch 1
Porcine skin	Porcine skin	Biological samples
Human skin	Human skin	Biological samples

1. *In vitro* permeation tests of caffeine gel through artificial and biological skin barriers

Permeation studies of caffeine gel formulation (2% w/w) were performed in Franz-type static diffusion cells using artificial barriers (PermeaPad® Skin barrier) and full-thickness human and piglet ear skin, according to OECD guidelines (OECD 2004). Artificial membranes, characterized by their polarized structure, were mounted with the paper filter side facing the donor compartment and the regenerated cellulose side facing the receptor. Similarly, human or porcine skin samples were mounted between the donor and receptor chambers with the *stratum corneum* facing the donor chamber. The effective diffusion area was 3.14 cm². The receptor fluid (RF) was composed of a freshly phosphate buffer saline (PBS) pH 7.4 continuously stirred using a Teflon coated magnetic stirrer. The receptor compartment had a mean volume of 15 mL filled with RF. Mounted

Franz cells were maintained at 32 ± 1°C. At time 0, infinite dose of 500 mg of gel formulation were applied directly onto the membrane surface, as well as on the porcine or human skin surface in the Franz cell. This resulted in a theoretical applied dose of Q_0 = 3.18 mg/cm². The donor compartment was sealed with parafilm during the whole time of the experiment. The permeation study was then carried out for 6h, in order to determine the permeation profile of caffeine remaining and permeating through the membrane or into the skin. At selected time points (0, 15, 30, 45, 60,120,180,240, 300, 360 minutes) 1.0 mL of each receptor sample was collected and analyzed. An equal volume of fresh receptor fluid was immediately replaced in each sample in order to maintain sink conditions. Porcine skin samples and biomimetic barriers were tested in triplicate, while human skin samples were tested in duplicate. At the end of the study (6 h), the amount of caffeine in the receptor fluid, as well as in each skin layer and within the artificial membranes, was quantified by HPLC.

1.1. Results and discussion

1.2. Permeation profile of caffeine gel through PermeaPad® Skin barrier, porcine skin and human skin

The concentration of caffeine measured in the receiving phase allows for the evaluation of the actual amount of the compound that crosses the membranes and can be systemically absorbed. The mean values are shown in Figure 1.

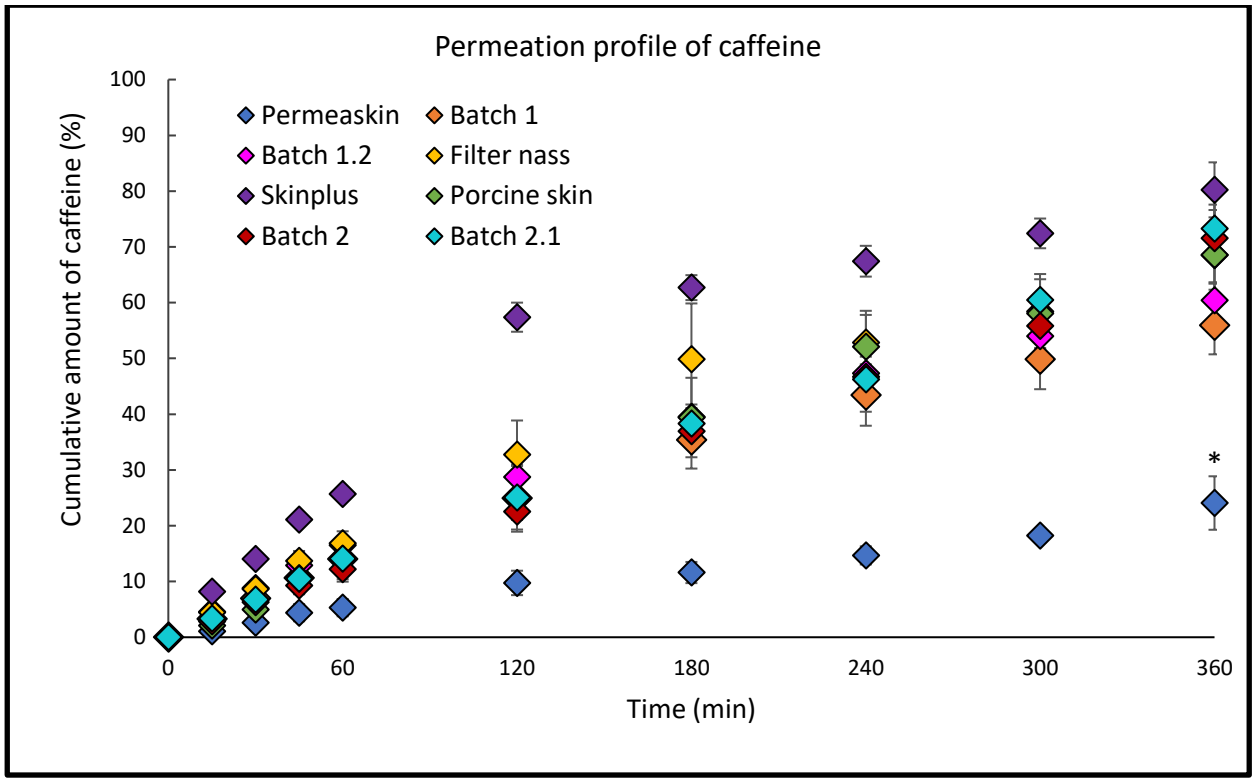

Figure 1: Permeation profile of caffeine gel formulation that permeated in the receptor fluid at specific extraction times through PermeaPad® Skin barriers and porcine skin. Values are expressed as mean ± standard error of the mean (SEM) (n=3). * indicates a statistically significant difference between porcine skin and the biomimetic PermeaPad® Skin Barrier membranes in a one-way ANOVA test ($p < 0.05$).

Table 1: Flux (J), Papp and resistance values of each membrane tested. Data are presented as mean ± SD (n = 3).

Membrane	J (mg/cm^2*s)	P_{app} (cm/s) ¥	Resistance (s/cm)
Permeaskin	$2.48 \times 10^{-5} \pm 0.17 \times 10^{-5}$	$1.50 \times 10^{-6} \pm 0.01 \times 10^{-5}$	$4.05 \times 10^{+4} \pm 2.80 \times 10^{+3}$
Batch 1	$9.58 \times 10^{-5} \pm 3.45 \times 10^{-5}$	$5.79 \times 10^{-6} \pm 0.21 \times 10^{-5}$	$1.16 \times 10^{+4} \pm 5.08 \times 10^{+3}$
Batch 1.2	$11.3 \times 10^{-5} \pm 1.17 \times 10^{-5}$	$6.84 \times 10^{-6} \pm 0.07 \times 10^{-5}$	$0.89 \times 10^{+4} \pm 0.93 \times 10^{+3}$
Batch 2	$10.3 \times 10^{-5} \pm 0.81 \times 10^{-5}$	$5.06 \times 10^{-6} \pm 0.05 \times 10^{-5}$	$1.17 \times 10^{+4} \pm 1.40 \times 10^{+3}$
Batch 2.1	$9.29 \times 10^{-5} \pm 0.69 \times 10^{-5}$	$5.45 \times 10^{-6} \pm 0.04 \times 10^{-5}$	$1.09 \times 10^{+4} \pm 0.82 \times 10^{+3}$
Batch Filter Nass	$10.8 \times 10^{-5} \pm 3.00 \times 10^{-5}$	$6.52 \times 10^{-6} \pm 0.18 \times 10^{-5}$	$0.98 \times 10^{+4} \pm 3.20 \times 10^{+3}$
Batch skin plus	$19.6 \times 10^{-5} \pm 4.99 \times 10^{-5}$	$11.8 \times 10^{-6} \pm 0.30 \times 10^{-5}$	$0.53 \times 10^{+4} \pm 1.20 \times 10^{+3}$
Porcine skin	$9.39 \times 10^{-5} \pm 0.09 \times 10^{-5}$	$5.67 \times 10^{-6} \pm 0.06 \times 10^{-5}$	$1.07 \times 10^{+4} \pm 0.11 \times 10^{+3}$

¥ P_{app} has been calculated assuming the full solubilization of all caffeine in the gel (2% w/w)

Table 2: The total absorbed amounts in RC, in the entire membrane and Q_{abs} of caffeine measured through each membrane tested. Values are expressed as percentage ± SD (n = 3).

Membrane	RC (%)	Total membrane (%)	Q_{abs} (RC+Total membrane) (%)
Permeaskin	24.59 ± 8.72	14.94 ± 0.96	39.53 ± 9.23
Batch 1	55.58 ± 9.25	6.51 ± 1.85	62.09 ± 7.49
Batch 1.2	60.22 ± 5.30	6.51 ± 1.61	66.73 ± 3.70
Batch 2	71.53 ± 9.21	8.17 ± 1.67	79.70 ± 7.53
Batch 2.1	73.27 ± 4.30	8.40 ± 0.97	81.68 ± 4.39
Batch Filter Nass	70.52 ± 14.4	6.79 ± 2.08	77.31 ± 11.7
Batch skin plus	83.80 ± 6.71	4.30 ± 0.24	88.10 ± 6.93
Porcine skin	70.57 ± 0.01	1.99 ± 0.80	72.59 ± 8.20
Human skin	0.00 ± 0.00	3.46 ± 0.57	3.46 ± 0.57

The results show that the amount of caffeine that permeated through all artificial membranes falls within the same range than those observed with porcine ear skin (Figure 1). All PermeaPad® Skin barriers display a positive correlation with porcine skin in terms of flux values, P_{app}, and resistance, highlighting the strong correlation between these artificial membranes and skin samples (Table 1). Indeed, there is no significant difference between Batch 1, 1.2, Filter nass, 2, 2.1 and the porcine skin, in terms of flux, P_{app}, and R, which is remarkable (Table 1, Figure 1). Moreover, slightly higher values were recorded with the PermeaPad® Skin barrier containing a higher amount of ceramides. This can be explained by the fact that caffeine is a small hydrophilic compound which has a very limited accumulation in the barrier, as it crosses rapidly the membrane, reaching the receptor fluid. However, it is important to note that caffeine could not be quantified in the receptor phase of human skin, suggesting that permeation was below the detection limit of the analytical method. Consequently, flux, P_{app}, and resistance could not be calculated for human skin. Regarding caffeine accumulation in the total membrane for each tested sample, we can observe that the mean amount of caffeine stocked in all PermeaPad® Skin Barrier membranes was slightly higher than those registered in porcine and huma skin (1.99 ± 0.80 % and 3,46 ± 0,57, respectively; Table 2). Similarly, higher values were recorded with the PermeaPad® Skin barrier containing a higher amount of ceramides such as batch 2, 2.1 and Skin Plus.

2. *In vitro* permeation tests of diclofenac gel through artificial and biological skin barriers

Permeation tests of diclofenac gel formulation (2% w/w) were performed following the same protocol described for caffeine (paragraph 1). For this study, automated Logan Instruments Corp. Franz cells were used, with an exposed surface area of 1.00 cm^2 and a mean receptor compartment volume of 12 mL. The theoretical applied dose (Q_0) was 10 mg/cm^2.

2.1. Results and discussion

2.2. Permeation profile of diclofenac gel through PermeaPad® Skin barrier, porcine skin and human skin

The concentration of diclofenac measured in the receiving phase allows for the evaluation of the actual amount of the compound that crosses the membranes and can be systemically absorbed. The mean values are shown in Figure 2.

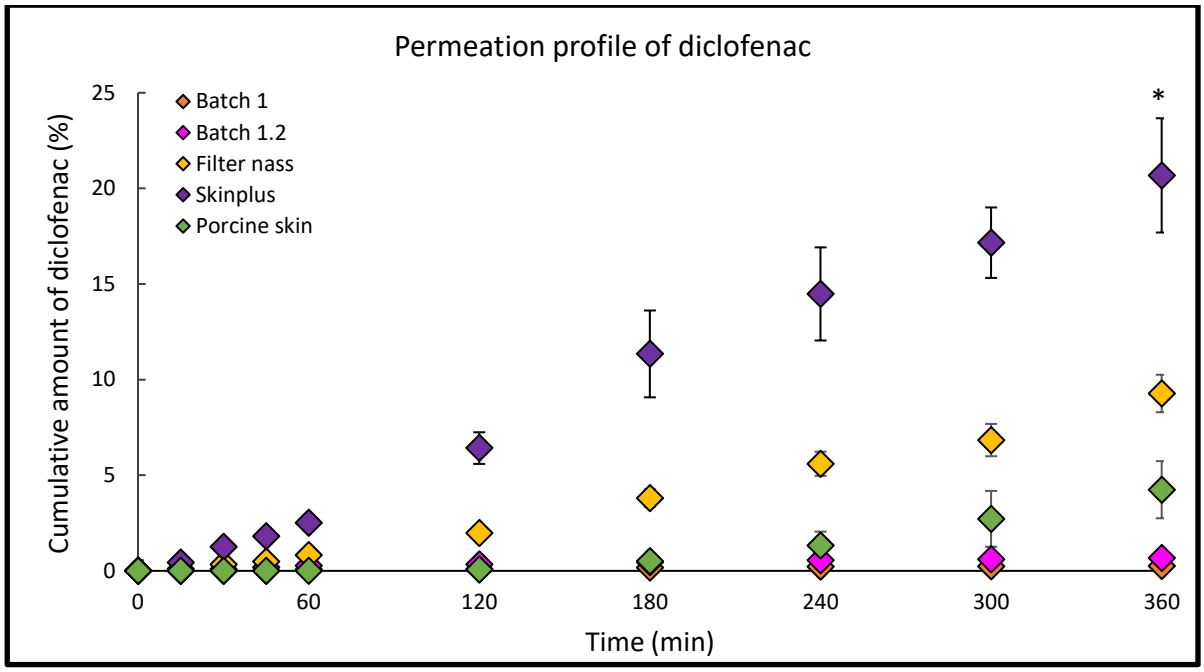

Figure 2: Permeation profile of caffeine gel formulation that permeated in the receptor fluid at specific extraction times through PermeaPad® Skin barriers and porcine skin. Values are expressed as mean ± standard error of the mean (SEM) (n=3). * indicates a statistically significant difference between porcine skin and the biomimetic PermeaPad® Skin Barrier membranes in a one-way ANOVA test ($p < 0.05$).

Table 3: Flux (J), Papp and resistance values of diclofenac for each membrane tested. Data are presented as mean ± SD (n = 3).

Membrane	J (mg/cm^2*s)	P_{app} (cm/s) ¥	Resistance (s/cm)
Batch 1	$0.77 \times 10^{-6} \pm 3.67 \times 10^{-7}$	$0.45 \times 10^{-7} \pm 0.21 \times 10^{-7}$	$167 \times 10^{+4} \pm 4.39 \times 10^{+5}$
Batch 1.2	$2.06 \times 10^{-6} \pm 3.10 \times 10^{-7}$	$1.20 \times 10^{-7} \pm 0.18 \times 10^{-7}$	$51.4 \times 10^{+4} \pm 0.93 \times 10^{+5}$
Batch Filter Nass	$11.8 \times 10^{-6} \pm 12.1 \times 10^{-7}$	$6.87 \times 10^{-7} \pm 0.71 \times 10^{-7}$	$8.70 \times 10^{+4} \pm 0.10 \times 10^{+5}$
Batch skin plus	$24.7 \times 10^{-6} \pm 19.0 \times 10^{-7}$	$14.4 \times 10^{-7} \pm 1.11 \times 10^{-7}$	$4.10 \times 10^{+4} \pm 0.03 \times 10^{+5}$
Porcine skin	$7.23 \times 10^{-6} \pm 21.9 \times 10^{-7}$	$4.22 \times 10^{-7} \pm 1.28 \times 10^{-7}$	$15.2 \times 10^{+4} \pm 0.46 \times 10^{+5}$

¥ P_{app} has been calculated assuming the full solubilization of all diclofenac in the gel (2% w/w)

Table 4: The total absorbed amounts in RC, in the entire membrane and Q_{abs} of diclofenac measured through each membrane tested. Values are expressed as percentage ± SD (n = 3).

Membrane	RC (%)	Total membrane (%)	Q_{abs} (RC+Total membrane) (%)
Batch 1	0.17 ± 0.09	12.47 ± 1.11	12.63 ± 1.20
Batch 1.2	0.67 ± 0.12	11.85 ± 1.03	12.52 ± 1.07
Batch 2	0.00 ± 0.00	13.75 ± 1.88	13.75 ± 1.88
Batch 2.1	0.00 ± 0.00	15.32 ± 0.78	15.32 ± 0.78
Batch Filter Nass	9.27 ± 0.98	13.24 ± 0.94	22.51 ± 1.51
Batch skin plus	20.68 ± 2.99	10.25 ± 1.25	30.93 ± 1.79
Porcine skin	4.24 ± 1.50	20.80 ± 1.52	25.04 ± 2.30
Human skin	0.00 ± 0.00	4.75 ± 0.82	4.75 ± 0.82

The results show that diclofenac permeation across the tested membranes was markedly lower compared to caffeine, with overall values about twenty times lower (Figure 2). Among the biomimetic membranes, Batch 1 and 1.2 displayed the closest profiles to porcine skin, with no significant differences in terms of permeation (Figure 2). On the other hand, permeation values were considerably higher for batch Skinplus in line with the

trend already observed with caffeine (Figure 2). Importantly, for human skin, and for batch 2 and 2.1 (higher lipid content) diclofenac could not be quantified in the receptor phase, indicating that permeation was below the detection limit of the HPLC method. Consequently, flux, P_{app}, and resistance could not be calculated for these samples. For this compound the amount of drug which was retained by the barrier was used for comparison. The diclofenac retention across all PermeaPad® Skin Barrier membranes was in the same order of magnitude of the one measured in porcine skin, and human skin, supporting the relevance of these models (Table 4). Importantly, no significant differences with the porcine skin were found, confirming the positive correlation between the artificial and biological models.

3. Conclusion

Overall, the study demonstrates a strong correlation between PermeaPad® Skin Barrier membranes and biological skin models, both porcine skin and, more importantly, human skin, for both caffeine and diclofenac. These findings support the applicability of PermeaPad® Skin Barrier membranes as reliable and reproducible alternatives to animal and human skin in permeation studies, with specific batches (e.g., Skinplus and batch 1.2) showing particularly strong alignment depending on the physicochemical properties of the tested compound. Batch 2 and 2.1 which retain diclofenac as much as human skin might be extremely biomimetic but the detection for lipophilic compounds in the acceptor might be difficult on classical franz diffusion cells.

Trieste, 29/08/2025

Prof. MASSIMILIANO PIO DI CAGNO

Dott.ssa GRETA CAMILLA MAGNANO