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In vitro and *in vivo* evaluation of drug release from semisolid dosage forms

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This study presents the *in vitro* and *in vivo* testing of anti-inflammatory drug containing creams, hydrogels and organogels for dermal use. *In vitro* penetration studies were performed with products by measuring the diffused drug amount through synthetic membranes soaked in isopropyl myristate (IPM). Our developed preparations were investigated under *in vitro* conditions together with two marketed medicinal products used as reference preparations. *In vivo* studies were carried out on anaesthetized male Wistar rats; the carrageenan-induced paw oedema decreasing effect of twelve different formulations and the reference products were measured in comparison with a control group. All - previously *in vitro* screened - selected products reduced paw oedema in rats. Significant differences were found among the developed products both *in vitro* and *in vivo*. Correlation between the *in vitro* penetration studies and *in vivo* results were found in the case of o/w creams, organogels and hydrogels.

1. Introduction

Properties of and drug release from semisolid preparations have recently found the interest of researchers from an application (Silva et al. 2009; Wu et al. 2010; Belal et al. 2011; Chen et al. 2011) and regulatory perspective (Csóka et al. 2007) as well as regarding their effects on the skin (Yamaguchi et al. 2006; Elsayed et al. 2007). Dissolution testing - which is frequently used in the examination of other dosage forms -, is also widespread in case of semisolid dosage forms (Sznitowska et al. 2004). Although many *in vitro* and *in vivo* methods are used for studying the penetration through the skin (Schmitt et al. 2010; Azeem et al. 2010; Hoffmann and Müller-Goymann 2005; Shakeel 2008, 2009; Shams et al. 2010), there are neither validated methods for their *in vitro* characterization in any Pharmacopoeias (USP 23/NF 18 1995) nor adequate *in vivo* sampling techniques for their investigation (Kanfer et al. 2010).

The aim of regulatory authorities is reducing the number of animal studies and precede their use with *in vitro* investigations. At present, *in vitro* studies are not required by regulators.

Methods for measuring percutaneous absorption are: studies with mathematical models, model membranes, stratum corneum, keratome slices, perfused or whole skin and *in vivo* techniques. Models for them can be: mouse, rabbit, rat, guinea pig, swine, primate and human. The confidence level is high in case of *in vivo* investigations and use of human skin. The physiological hierarchy is increasing with decrease of hairless strains. From the above mentioned list we chose the model membrane and *in vivo* study for our investigation (Howes et al. 1996).

In vitro tests can be used for screening the compositions prior to *in vivo* animal testing, although there are many anatomical and physiological factors, which are not properly represented under *in vitro* conditions (Barry 1983; Naegel et al. 2008).

There have been several methods to predict drug penetration in humans with human (Tadini and Maiai Campos 2009; Dragievic-Curic et al. 2010) and animal *in vitro* models, although animal skin (mouse (Wasdo et al. 2009; Heo et al. 2010), rat (Shakeel et al. 2008; Melero et al. 2010), pig (Songkro et al. 2009; Caon et al. 2010), guinea pig (Barbero and Frasch 2009; Doan et al. 2010), rabbit (Nicoli et al. 2006; Meshali et al. 2008), snake skin (Haigh et al. 1998; Ngawhirunpat et al. 2006)) tend to be more permeable than human. Rat abdominal skin (Farahmand and Maibach 2009; Kumar et al. 2009) has been shown to be a reasonable model based on the *in vitro* static cell experiments.

Guidelines offer as methodology for these studies the Franz diffusion cell (FDC) which is frequently used (Franz 1975; Fasolo et al. 2009; Kim et al. 2009; Ogita et al. 2010).

Fundamental requirement for these studies is the *in vitro-in vivo* correlation (IVIVC) (Wissing and Müller 2002; Buch et al. 2010) posing a major challenge not only for solid, but also for semisolid dosage forms (FDA Guidance 1997 and 1998; Shah 2005; Cardot et al. 2007; Retting and Mysicka 2008).

The purpose of this study was to evaluate developed compositions both *in vitro* and *in vivo* (Medeiros et al. 2009) and to assess whether there is an *in vitro-in vivo* correlation or not.

Despite the increasing number of publications within this field, no standard experimental conditions are used, therefore the comparison of the data is very difficult.

2. Investigations and results

Because of high variability among *in vitro* skin techniques and different data with high standard deviation measured with FDC, we investigated the *in vitro* and *in vivo* characteristics and *in vitro-in vivo* correlation of our products. A simple isopropyl myristate (IPM) model was chosen to simulate the partition phenomenon, which occurs when absorbing through the outermost layer of the skin. It is a question, whether the usage of IPM under *in vitro* circumstances can help in screening prior to *in vivo* testing (Barry 1983; Thakker and Chern 2003).

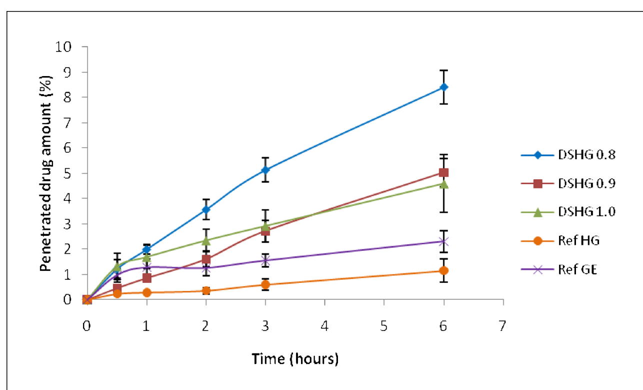

Fig. 1: Cumulative penetrated diclofenac sodium in hydrogels through IPM soaked synthetic membrane

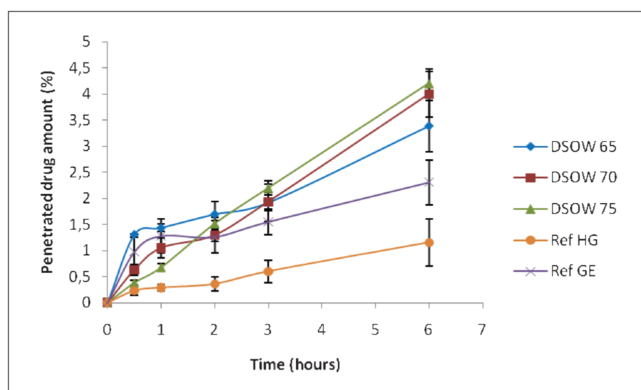

Fig. 3: Cumulative penetrated diclofenac sodium in o/w creams through IPM soaked synthetic membrane

In our study the suitability of an *in vitro* methodology for predicting *in vivo* performance was studied. Diclofenac sodium (DS), a non-steroidal anti-inflammatory agent was chosen as a model drug (Aurora-Prado et al. 2002; Lopes et al. 2003).

When developing these compositions, we focused on a less number of additives sensitizing the skin and also using them at lower concentrations to extend the present product range. These developed products were compared to the reference products in efficiency.

Our hypothesis was, that *in vitro* penetration studies carried out by means of IPM can be used as a surrogate of different animal skin models and brings us closer to the proper *in vitro* selection of products for *in vivo* studies. Results of the *in vitro* penetration were compared, and correlation between these results and the *in vivo* performance were observed.

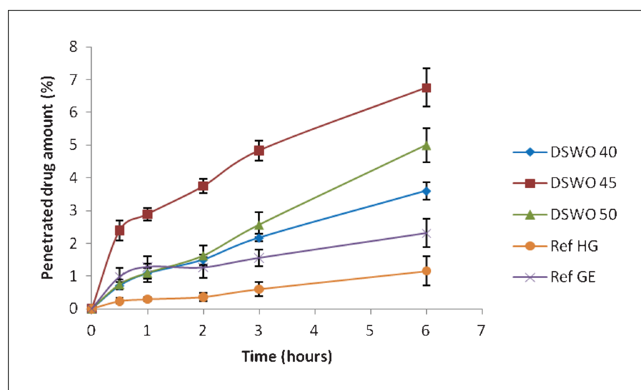

Fig. 4: Cumulative penetrated diclofenac sodium in w/o creams through IPM soaked synthetic membrane

2.1. *In vitro penetration study of diclofenac sodium*

The penetration process from different vehicles (n = 12) and two reference gels were measured through synthetic cellulose acetate membrane soaked in IPM.

Figures 1–4 and Table 1 show the penetrated diclofenac sodium amount in percentage against time through IPM soaked membrane. *In vitro* penetrated drug amount during a given time period was 6.01% from hydrogel formulations, 5.17% from organogels, 3.63% in the case of o/w creams and 5.12% from w/o creams in average. All of our products reached the drug penetration level of reference gels (penetrated drug amount of reference hydrogel was 1.16% and reference gelemulsion was 2.31%). The hydrogel samples containing 0.8% polymer showed the highest *in vitro* penetration rate (8.41%) and the reference hydrogel was the last in the order. Standard deviation (SD) were in the range from 0.46% (DSOG 25) to 2.29% (DSHG 1.0).

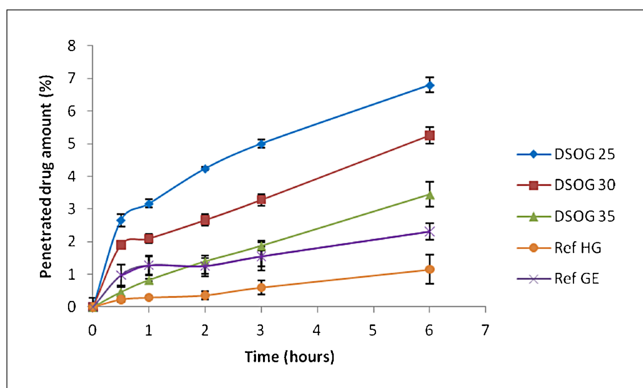

Fig. 2: Cumulative penetrated diclofenac sodium in organogels through IPM soaked synthetic membrane

2.2. *In vivo percutaneous testing of diclofenac sodium*

A carrageenan-induced oedema study was used to test *in vivo* efficiency of formulations Data are presented in Fig. 5. Diclofenac sodium 1% (w/w) in 40%, 45% w/o creams ($p < 0.05$) (labelled with *) exerts only a moderate oedema inhibition compared to the control group. One percent (w/w) active agent incorporated in 50% w/o cream, in the o/w cream vehicles, in hydrogel and organogel preparations and in case of both marketed reference products showed to be efficient in comparison with the non-treated group ($p < 0.001$) (labelled with ***). The highest oedema swelling inhibition rate was measured in case of

Table 1: Cumulative penetrated diclofenac sodium measured within 6 h in case of IPM soaked membrane

Composition	Penetrated drug amount ± SD (%)
DSHG 0.8	8.41 ± 1.34
DSHG 0.9	5.03 ± 1.12
DSHG 1.0	4.60 ± 2.29
DSOG 25	6.8 ± 0.46
DSOG 30	5.25 ± 0.51
DSOG 35	3.45 ± 0.77
DSOW 65	3.39 ± 0.99
DSOW 70	4.00 ± 0.86
DSOW 75	4.20 ± 0.54
DSWO 40	3.60 ± 0.54
DSWO 45	6.75 ± 1.17
DSWO 50	5.00 ± 1.05
Ref HG	1.15 ± 0.91
Ref GE	2.31 ± 0.86

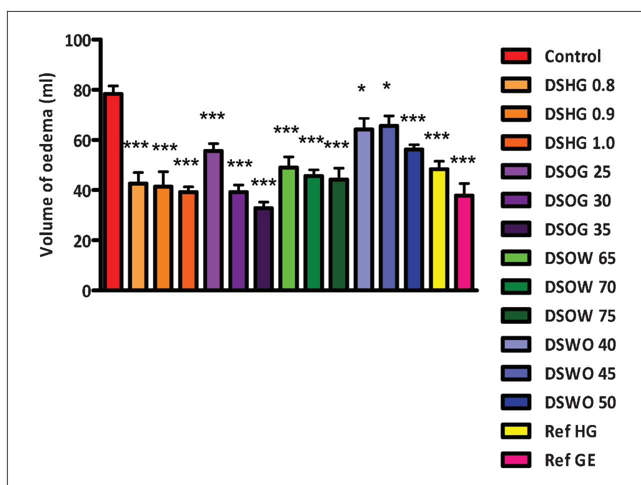

Fig. 5: Anti-inflammatory effect of different preparations containing 1% diclofenac sodium on carrageenan-induced oedema in rats

the 35% emulsifier containing organogel, which was more significant than both of the registered products. The lowest effect was observed in the 45% water containing w/o formulation.
More than 58% of our products reached or exceeded the oedema decreasing effect of the reference hydrogel, and 8% of our formulations exceeded the value of the reference gelemulsion.

2.3. *In vitro-in vivo correlation (IVIVC)*

Moderate (x < 0.90), significant (0.90 < x) or no correlation at all were measured between the *in vitro* penetration results compared to that of *in vivo* efficiency studies with difference fitting. Best IVIVC was established in case of IPM soaked membrane in o/w creams and in organogel samples in case of linear fitting and in o/w creams in case of power trend line fitting. IVIVC rate was not remarkable in w/o samples (Table 2). It can be concluded, that o/w creams had the best (0.9732 and 0.9714) and w/o creams the worst fitting (0.0403 and 0.0228) also in case of linear and power trend line fitting.
Based on evaluation of absolute values between *in vitro* penetration and *in vivo* efficacy studies the 0.9% hydrogel, the 30% organogel and the 40% w/o cream had the best IVIVC order numbers and the reference gel emulsion had the worst order number. IVIVC was also established in case of 0.8% and 1.0% hydrogel, 65%, 70%, 75% o/w creams and in 50% w/o cream. Correlation in reference gels was not significant. No correlation was found between the *in vitro* penetration study and *in vivo* results in case of reference gels.
The reference hydrogel was compared to the 0.9% hydrogel. *In vitro* penetration order number studies showed, that our product had better values than the reference hydrogel (Table 3).
The reference gel emulsion was not compared with gel emulsion sample, because in earlier studies their *in vitro* drug release rate (results under publication, not published here) proved to be the lowest among our developed products.

Table 2: IVIVC correlation coefficients in case of IPM soaked membrane with linear and power trend line fitting

Composition	R^2 with linear fitting	R^2 with power trend line fitting
Hydrogel	0.6913	0.7331
Organogel	0.9176	0.81
O/w cream	0.9732	0.9714
W/o cream	0.0403	0.0228

3. Discussion

Impregnation of membranes with IPM for *in vitro* penetration studies was already analysed previously (Pénzes et al. 2005). It was shown that different natures of model barriers (synthetic cellulose acetate membrane and stratum corneum) used in the experiment resulted in significant differences in the *in vitro* penetration and *in vivo* absorption. The synthetic cellulose acetate membrane is an inert barrier, while skin is an active barrier. Although the rate limiting step of diffusion through the skin is the stratum corneum which consists of dead cells. That is why we employed the reliability of diffusion through the stratum corneum with IPM soaked membrane in order to mimic a lipophilic barrier.
In vitro penetration studies were performed in order to evaluate the effect of IPM. Diffusion through the IPM soaked membrane decreased in the following order: hydrogels > organogels > w/o creams > o/w creams > reference gel emulsion > reference hydrogel. Based on the *in vitro* penetration results many products could be excluded because of their low penetration rate, although they were effective in the *in vivo* studies. This questions the applicability of IPM for screening.
Several studies examined the paw oedema decreasing effect of different compounds (Blazsó and Gábor 1997; Szabados-Nacsa et al. 2011). The paw oedema decreasing effect of our products (n = 12) and two reference gels were also investigated in our work. Similar to a previous study (Csóka et al. 2005) - where *in vitro* and *in vivo* percutaneous absorption of ketamine hydrochloride and piroxicam were investigated -, we also found, that our hydrogel samples showed to be efficient *in vitro* and *in vivo*. In contrast to this investigation, in our experiments *in vitro-in vivo* correlation was found among our o/w cream, organogel and hydrogel samples.
In the *in vivo* experiments the average order of the preparations was as follows: reference gel emulsion > hydrogels > organogels > o/w creams > refrence hydrogel > w/o creams. More than half of our developed products reached and exceeded the oedema decreasing effect of the reference hydrogel, and one preparation exceeded the value of reference gel emulsion. It means that our aim to develop new products with fewer additives, that reach the oedema decreasing effect of reference gels, was successful.
All the developed products with less additives than the reference gels, except the 25% organogel, 65% o/w cream and w/o compositions, reached minimum the same *in vivo* effect as the reference hydrogel. The anti-inflammatory effect of one formulation (DSOG 35) was higher than the reference gel emulsion. However, the *in vitro* penetration and *in vivo* results showed significant differences in the dosage form types and even within each group of dosage forms. Correlation was found between the *in vitro* penetration and *in vivo* data of o/w creams and organogels and moderate IVIVC was found in case of hydrogels.
In the guidelines edited by the FDA and in cooperation of International Pharmaceutical Federation (FIP) and American Association of Pharmaceutical Scientists (AAPS), the Franz vertical diffusion cell is accepted as a ‘gold standard’ method for semisolid investigations (FDA Guidance for Industry SUPAC-SS 1997; Siewert et al. 2003). Similar to the profile of these guidelines, the FDC was found to be proper for prediction of *in vivo* results (Tashtoush et al. 2004). However many critical points were observed in connection with the methodology itself; the very low amount of receiving medium, the complicated tube system, and the possibility of bubble formation emphasises the necessity to validate the method (results under publication, not published here).
Implementation of IPM as a “skin model” as a surrogate for different animal skin models can be used only together with release data. The release results are handled as the drug diffuses 100%

Table 3: *In vitro-in vivo* number according to *in vitro* penetration and *in vivo* oedema decreasing effect

Composition	*In vitro number*	*In vivo number*	Absolute value of *in vitro-in vivo* difference	Order number of absolute value
DSHG 0.9	5	4	1	1
DSOG 30	4	3	1	1
DSOW 75	8	6	2	2
DSWO 40	10	11	1	1
Ref HG	14	7	7	4
Ref GE	13	2	11	10

through the polar channels, partitioning from IPM which represents the 100% lypophilic way of absorption through the stratum corneum. The *in vivo* behaviour will be between these data as the consequence of both polar and apolar ways of absorption. Our conclusion is, that hydrophilic preparations diffused more readily through synthetic membranes, while an opposite order is experienced in case of penetration studies.
We strengthen the fact, that penetration studies are not acceptable without *in vivo* feedback. But what type of *in vivo* studies can be accepted? Which one gives a good prediction for clinical use? Differently permeable animal skin investigated under very different circumstances (pH, temperature, methodology, thickness) or even cadaver human skin or a simple physicochemical model (like IPM) showing no interindividual variability will give a good prediction.
In the present study IVIVC with the animal tests in use was found in some cases. Evaluating the *in vitro*, *in vivo* and IVIVC data we can offer our hydrogel and organogel compositions for clinical use.
Our developed products were able to show anti-inflammatory effects and to decrease the carrageenan-induced oedema in rats.

4. Experimental

4.1. *Materials*

Micronized diclofenac sodium (DS) (Ph.Eur. 6) was used as hydrophilic active ingredient (Human Co., Hungary). The following materials were used as vehicle components. Carbomer 934P (prop-2-enoic acid) was obtained from BF Goodrich, Brussels, Belgium. Sorbitan monopalmitate (Span 40) was purchased from Sigma-Aldrich, Hungary. Miglyol 812 N (fractionated coconut oil, glyceryl tricaprylate/caprate) and Imwitor 780K (isostearyl diglyceryl succinate) was donated by Sasol, Germany. Pemulen (PTR-2) was a gift from Novean, USA. Tagat S (PEG-30 glyceryl stearate) was supplied by Evonik, Germany. Isopropyl myristate 98%, disodium hydrogenphosphate and citric acid were ordered from Merck, Germany. All other additives, triethanolamine, liquid paraffin, cetostearyl alcohol and sodium hydroxide were purchased from Hungaropharma Co., Hungary. All components used were of Ph.Eur. 6 grade. The reference hydrogel (Ref HG) and the reference gel emulsion (Ref GE) are commercial medicinal products.
Table 4 summarizes the compositions selected and investigated in this study. The concentration of DS used in the formulations was 1% (w/w) and it was suspended in the vehicles. The numbers used in each coded sample series indicate the concentration of a particular ingredient of the formulation, as follows: hydrogel (HG) contains 0.8, 0.9 and 1.0% Carbomer 934P; organogel (OG) contains 25, 30 and 35% Span 40 emuslifier; gel emulsion includes 40,45 and 50% Pemulen TR-2 gel; oil in water (O/W) cream consists of 65, 70 and 75% purified water; water in oil (W/O) cream includes 40, 45 and 50% purified water.

4.2. *Preparation of the formulations*

In case of hydrogels, Carbopol 934 P was used as polymer and it was added first to purified water. Constant stirring at room temperature at 450 rpm - 625 rpm (Stuart heat-stir, England, Sterilin Ltd.) was continued until the complete dissolution of the powder. It was followed by adding triethanolamine until the three-dimensional network was built up and the pH of the sample was adjusted to 7.0 (ISFET pH Meter, IQ Scientific Instruments, Inc., USA) to form a neutralised clear gel. The organogel samples were produced by melting (80 °C) sorbitan monopalmitate and Miglyol 812 N oil together under continuous stirring and homogenization.
Cetostearyl alcohol, liquid paraffin and Tagat S were melted together (80 °C) and mixed in order to prepare the oil phase of the oil-in-water cream preparations. The aqueous phase containing purified water was heated up to a similar temperature. The phases were mixed and homogenized until the cream cooled down to room temperature.
In water-in-oil creams cetostearyl alcohol, liquid paraffin and Imwitor 780 K were melted together (80 °C) and mixed. Purified water was heated up to similar temperature, mixed with oil phase, homogenized and cooled down to 25 °C.
The hydrogel and organogel samples were stored at cold temperature (10 °C) for 1 day before adding the active agent. The o/w and w/o creams were stored at the same temperature written above (already containing the active agent) for 1 day before testing.

4.3. *In vitro penetration study of diclofenac sodium*

A franz vertical diffusion cell system (Hanson Research Co., USA) containing six cells and equipped with autosampler (Hanson Microette Autosampling System, USA) was used. The area for diffusion was 1.767 cm^2 and the receptor chamber volume was 7 ml. Cellulose acetate membranes (Porafil, Macherenay-Nagel, Germany and Pall Life Sciences, USA) with an average pore size of 0.45 μm were used. Membranes were soaked in isopropyl myristate for *in vitro* penetration studies to mimic a lypophilic barrier like the stratum corneum (Shas et al. 1991). Membrane filters were mounted on the top of the Franz diffusion cells. A stirring rate of 450 rpm was used. The dissolution medium temperature was maintained at 32 ± 0.5 °C. Phosphate buffer (pH 5.4 ± 0.1) (Orion Star pH, Thermo Electron Co., Singapore) was chosen as receiving medium as representative of the physiological values of dermis and skin surface. On one hand, our aim was to validate our *in vitro* drug release results (results under publication, not published here). In the drug release investigations the receiving medium pH was pH 5.4 ± 0.1. In order to compare the *in vitro* drug release to the penetration results, pH was set the same as in our previous study, because in the validation method experiment conditions can not be changed. Our first examinations were carried out with 5.4 pH. In the future we plan to study the penetration results with 7.4 pH, because the physiologically conductive fluid usually used is phosphate buffer pH 7.4 (Barry 1983).
The receptor medium allows sufficient amount of active ingredient released within a reasonable time period to ensure accurate analysis. Diclofenac sodium was chosen as an active agent. On the other hand, diclofenac sodium is poorly soluble in acidic (pH 1-3), but is rapidly soluble in alkaline conditions (pH 5-8) (Tripathi 1998; Manjunatha et al. 2007). That is why our first step was to maintain alkalic (pH 5.4) conditions in this study.
Samples (0.24–1.65 g, amount depended on types and consistency of the vehicles) of different compositions were placed evenly on the surface of the membrane, and 800 μl samples were taken after 0.5, 1, 2, 3 and 6 h and replaced with fresh receiving medium. The absorbance of the diclofenac sodium content was measured by UV Spectrophotometer (Unicam Helios α UV–Vis Spectrophotometer, England) at 275 nm, based on prior calibration curve. The blank vehicles without active agents served as references in the analytical measurements. No sink conditions were used.
Four parallel measurements were done with plotting the penetration amount of diclofenac sodium in percentage over a 6 hours time period. Results were expressed as the mean ± S.D.

4.4. *In vivo percutaneous testing of diclofenac sodium*

4.4.1. *Experiments*

Products for *in vivo* testing were selected based on *in vitro* results (results under publication, not published here). Experiments were approved by the Animal Ethics Committee of the University of Szeged, Hungary (IV/01758-6/2008).
Male Wistar rats (150–181 g) were studied. All measurements were performed at 24 ± 1 °C in an air-conditioned room. The animals were kept under standard 12 h light/12 h dark conditions with food and water *ad libitum*. All experiments were carried out in the same period of the day (1–4 p.m.) to

Table 4: Composition of formulations (%, w/w)

Component ↓ / Marking →	Hydrogel (HG) DSHG 0.8 DSHG 0.9 DSHG 1.0	Organogel (OG) DSOG 25 DSOG 30 DSOG 35 (w/w %)	o/w cream (O/W) DSOW 65 DSOW 70 DSOW 75	w/o cream (W/O) DSWO 40 DSWO 45 DSWO 50
Liquid paraffin	–	–	15–10–5	45–40–35
Triethanolamine	quantum satis	–	–	–
Miglyol 812 N	–	75–70–65	–	–
Imwitor 780 K	–	–	–	5
Tagat S	–	–	10	–
Carbopol 934 P	0.8–0.9–1.0	–	–	–
Span 40	–	25–30–35	–	–
Cetostearyl alcohol	–	–	10	10
Purified water	Ad 100	–	65–70–75	40–45–50

exclude diurnal variations in pharmacological effects. Each rat was tested only once. One day prior to the application of the preparations, the back of each rat (15 cm^2) was carefully shaven and depilated by Veet® depilatory cream (Reckitt Benckiser, France) in 5 min under 2.5–3.5% isoflurane anaesthesia (Forane® solution, Abbott Laboratories, Hungary). The skin of the animals was cleaned by wiping with water containing cotton. The rats were dried under infrared lamp for 10 min.
On the day of the experiment, the animals were anaesthetized with Forane® solution. Experimental animals were exposed to different vehicles (hydrogels, organogels, o/w and w/o creams) containing 1% (w/w) diclofenac sodium and to the two reference gels. Each formulation (300 mg) was applied onto the depilated dorsal skin of the rat. One group (n = 5) served as absolute control – it was not treated at all. The 12 remaining groups (n = 60) were treated with different vehicles containing diclofenac sodium, and 2 groups (n = 10) treated with commercial preparations (reference products). Local inflammatory response was elicited by 0.1 ml subplantar injection of carrageenan (Viscarin, Marine Colloids Inc., Springfield, USA) solution given into the right hand paw one hour after the treatment. The concentration of carrageenan solution was 0.5% which was prepared in physiological saline solution. The left paw, used as control, was treated without carrageenan (Gábor 2000). Paw volume was measured with a plethysmometer (Hugo Sachs Elektronik, Germany) 5 h after the injection.
The volume difference between the carrageenan- and saline-injected paws was used for the evaluation of the inflammatory response. The degree of paw swelling was calculated as:

$$\text{Swelling}\,(\%) = \frac{Vi - V}{V} \times 100 \qquad (1)$$

where Vi is the volume of the carrageenan-treated paw, V is that of the non-treated paw.
On the basis of Eq. (1), the percentage of oedema inhibition was calculated as:

$$\text{Inhibition}\,(\%) = \left(\frac{1 - \text{swelling}_{\text{treated}}}{\text{swelling}_{\text{control}}}\right) \times 100 \qquad (2)$$

Where $\text{swelling}_{\text{treated}}$ is the mean value observed in the treated group, and $\text{swellingt}_{\text{control}}$ is the mean value observed in the control group.

4.4.2. In vivo data analysis

Statistical analysis was performed by one-way ANOVA, followed by Newman-Keuls Multiple Comparison Test. At a significance level of $p < 0.05$, the anti-inflammatory effect was titled moderate, and at a significance level of $p < 0.001$ it was called significant (GraphPad 4.0). Data are presented as means ± S.E.M.
The relative bioavailability (RBA) regarding the systemic effect was calculated as:

$$\text{RBA} = \frac{\text{inhibition}\%_{\text{D}}}{\text{inhibition}\%_{\text{T}}} \qquad (3)$$

where $\text{inhibition}_{\text{D}}$ is the percentage oedema inhibition for the different D samples and $\text{inhibition}_{\text{T}}$ is the percentage oedema inhibition for the different T samples.

4.5. *In vitro-in vivo correlation*

Correlation rate was calculated between the *in vitro* penetration and *in vivo* absorption data.
Although a good correlation is a tool for predicting *in vivo* results based on *in vitro* data (Cardot et al. 2007), in case of semisolid dosage forms this correlation is not well established.
Linear and power trend line fitting between *in vitro* penetration data and *in vivo* absorption studies were used.
Correlation rate was evaluated as "good" above the coefficient rate of 0.90. The correlation was "moderate" below this value. No correlation was established when numbers were close to 0. Although many studies reported about basic concept of IVIVC, different methodologies (absorption studies, plasma concentration, AUC) are available based on the type of data (Cardot et al. 2007).

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