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Research paper

Effects of various vehicles on the penetration of flufenamic acid into human skin

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Abstract

The effect of various vehicles (polyacrylate gels and wool alcohol ointments) on the penetration of flufenamic acid into excised human skin was investigated. Physico-chemical properties of the formulations were examined and discussed. Penetration data was gathered using two different in vitro test systems: the Saarbruecken penetration model (SB-M) and the Franz diffusion cell (FD-C). With wool alcohol ointments, drug concentration in the formulation was the decisive parameter for drug liberation and penetration. The incorporation of water into wool alcohol ointment led to increased drug amounts within the deeper skin layers (DSL), especially after longer incubation times. The drug concentration within the stratum corneum (SC) was not influenced by the bleeding effect of lipophilic, liquid components of the various wool alcohol ointments. With polyacrylate gels different results for liberation and penetration were observed. These results could be related to the effects of the drug concentration within the formulation and the penetration enhancers incorporated into the gels. Especially the effects of penetration enhancers clearly illustrated that liberation experiments do not predict the situation in the skin, but make experiments with a biological barrier essential. The high water content of the gels led to hydration of the skin specimen for the SB-M and the FD-C and therefore, in contrast to previous findings, comparable data were obtained in the penetration studies with both models. Furthermore, the quasi steady-state drug amount in the SC could be calculated for all formulations using an equation derived from a Michaelis–Menten kinetics. The data from both test systems were linearly correlated to each other. In addition, a direct linear relationship between the SC drug amount and the drug amount in the DSL was found as long as the quasi steady-state drug amount in the SC was not reached. A combination of all results might offer the chance to reduce the costs and to simplify the development of a new drug formulation.

Keywords: Flufenamic acid; Saarbruecken penetration model; Franz diffusion cell; Human skin; Polyacrylate gels; Wool alcohol ointments

1. Introduction

Flufenamic acid is a non-steroidal anti-inflammatory drug of the anthranilic acid group with potent anti-inflammatory and analgesic effects mediated through the inhibition of prostaglandin synthesis. This mode of action is responsible for its therapeutic use as well as the negative side effects, e.g. gastrointestinal problems. Different products containing flufenamic acid are available on the market and are administered either via the peroral route or via the dermal route. Since the dermal route normally reduces the gastrointestinal side effects, it has been recognized as a popular alternative to the oral delivery of drugs. In addition, the cutaneous application of drugs combines the advantage of a local drug therapy with reduced first pass metabolism [1]. However, the stratum corneum (SC) is known to represent the major permeability barrier of the skin. To control the delivery of drugs into and across the skin the following strategies are possible:

1. Reducing the skin resistance as a penetration barrier by disrupting the tightly packed lipid bilayers of the SC.

Abbreviations: SC, stratum corneum; DSL, deeper skin layers; SB-M, Saarbruecken penetration model; FD-C, Franz diffusion cell; WAO, wool alcohol ointment; AWAO, aqueous wool alcohol ointment; APAG, aqueous polyacrylate gel; PPAG, propylene glycolic polyacrylate gel; IPAG, isopropanolic polyacrylate gel.

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2. Shifting the partitioning of the drug from the vehicle to the skin.
3. Delivering additional solvent to the skin, which leads to an additional convectional transport of the molecules or which enhances the drug solubility within the skin.

The objectives of this study were to examine the effect of hydrophilic and lipophilic vehicles, e.g. polyacrylate gels and wool alcohol ointments, on the in vitro penetration of flufenamic acid into excised human skin. For this purpose, two different experimental set-ups were used, namely the Franz diffusion cell (FD-C) and the Saarbruecken penetration model (SB-M). After predetermined periods of incubation the drug amount in the SC and the deeper skin layers (DSL) was analysed [method compare 2]. Furthermore, the physico-chemical properties of the drug preparations were characterised, e.g. concentration of drug at saturation within the liquid phase, bleeding, spreading and drug liberation without a biological barrier.

2. Materials and methods

2.1. Materials

The following materials and equipment were used: Flufenamic acid (Kali-Chemie Pharma, Hannover, D); wool alcohol ointment and Multifilm kristall-klar (Beiersdorf, Hamburg, D); Ringer solution, McIlvaine citric acid-phosphate buffer (pH 2.2), NaOH, Carbopol 980, propylene glycol and isopropanol (all components from Merck, Darmstadt, D); Plastibase® (Heyden GmbH, Muenchen, D); methanol (Baker, Deventer, NL); cryomicrotome HR Mark II, model 1978 (SLEE, Mainz, D); teflon filter Minisart, pore size 0.2 μm (Sartorius, Goettingen, D); isocratic HPLC consisting of a 655 A 40 autosampler, L 4250 detector, L 6220 pump, 6000 K data interface and 5 μm LiChrospher® 100/RP-18 column/12.5 cm × 4 mm (Merck-Hitachi, Darmstadt, D); light microscope, Olympus BH 10 (Olympus, Hamburg, D); Eppendorf-cetrifuge (Eppendorf, Hamburg, D).

The non-steroidal drug flufenamic acid, dissolved in wool alcohol ointment and suspended in polyacrylate gels (German Pharmacopoeia 1999), was used under 'infinite dose' conditions (Table 1; [3,4]). Before their application, the water free drug preparations were stored at 32 °C for one week to allow complete dissolution of the drug in the ointment base. This was checked by light microscopy. The water containing formulations were only incubated for one day at 32 °C.

2.1.1. Skin samples

Excised human skin from Caucasian female patients who had undergone abdominal plastic surgery was used. This was approved by the Ethical Commitee of the Caritas-Traegergesellschaft, Trier, Germany (6th July 1998). Immediately after excision the subcutaneous fatty tissue was removed using a scalpel. The skin was cut into 10 × 10 cm pieces, wrapped in aluminium foil and stored in polyethylene bags at −26 °C until use. The maximum storage time was 6 months. Previous experiments in our lab have shown that no changes in the penetration characteristics occur during this time, which is in accordance with findings of other labs [5–7].

2.2. In vitro skin experiments

For detailed descriptions of the experimental set-ups the reader is referred to [2].

2.2.1. Saarbruecken penetration model

Using the Saarbruecken penetration model (SB-M), a 2 mm ointment layer was put on the surface of the skin under standardised conditions. The skin was laid on a filter paper soaked with Ringer solution to prevent water loss during the experiment. The whole arrangement was sealed with Plastibase®, transferred into a plastic box and stored in a water bath at 32 ± 1 °C for different time periods. Afterwards the skin was segmented horizontally into different layers.

2.2.2. Franz diffusion cell

In the Franz diffusion cell (FD-C), excised skin samples were positioned between the donor compartment, containing the drug preparation, and the acceptor compartment

Table 1
Composition and properties of the drug formulations (each characterisation with $n = 3$; mean ± SE)

Drug formulation	Condition of the drug	Liquid phase	Hydrophil–lipophil	Conc. of saturation in the two phases (hydr./lip.) (%; w/v)	Bleeding number (cm^2)	Spreading (cm^2)
WAO; 0.45%	Dissolved	–	Lipophil	–/0.95 ± 0.04	52.00 ± 1.24	27.44 ± 4.59
AWAO; 0.45%	Dissolved	Water	Lipophil	0.0047 ± 0.0003/0.95 ± 0.04	26.12 ± 0.32	24.85 ± 3.05
WAO; 0.90%	Dissolved	–	Lipophil	–/0.95 ± 0.04	50.27 ± 0.58	29.60 ± 1.88
APAG; 0.45%	Suspended	Water, pH 6.3	Hydrophil	0.0129 ± 0.0004/ –	–	85.41 ± 5.79
PPAG; 0.45%	Suspended	Propylene glycol/water pH 5.4	Hydrophil	0.0073 ± 0.0001/–	–	67.25 ± 5.13
IPAG; 0.45%	Suspended	Isopropanol/water pH 5.4	Hydrophil	0.1959 ± 0.0129/–	–	84.55 ± 1.74

WAO: cetylstearyl alcohol 0.5%; wool alcohols 6.0%, white petrolatum 93.5%. AWAO: WAO 50.0%, water 50.0%. APAG: polyacrylic acid 0.5%, 1N NaOH solution 3.75%, water 95.75%. PPAG: polyacrylic acid 0.5%, 1 N NaOH solution 1.25%, propylene glycol 25.0%, water 73.25%. IPAG: polyacrylic acid 0.5%, 1 N NaOH solution 1.25%, isopropanol 25.0%, water 73.25%.

filled with Soerensen phosphate buffer at pH 7.4. During the experiments, the FD-C was kept at 32 ± 1 °C by a water jacket. The acceptor fluid was mixed using a magnetic stirring bar at 500 rpm. The skin was prehydrated with the basolateral receptor medium for 30 min. Samples from the acceptor medium were drawn from the middle of the acceptor compartment before and at the end of each experiment. Afterwards, the skin was segmented horizontally into different layers.

2.2.3. Horizontal segmentation of the skin

To compare the penetration data with either experimental set-up, the skin was always treated in exactly the same way. First, the remaining ointment was removed by wiping the skin with cotton. Second, the skin was transferred into a special apparatus where it was mounted on cork discs using small pins, and covered with a teflon mask. The skin was successively stripped with 20 pieces of adhesive tape that were placed on the central hole of the teflon mask. In a standardized procedure, each of the 20 tapes (size = 15 × 20 mm) was charged with a weight of 2 kg for 10 s and then removed rapidly. The tapes were combined in 6 pools of 1, 1, 3, 4, 5 and 6 strips for analytical purposes. The first tape strip was always discarded because of potential contamination.

After the tape stripping, the skin was quickly frozen in a stream of expanding carbon dioxide, and a specimen with a diameter of 13 mm was taken out of the stripped area and transferred into a cryomicrotome. The skin was cut into sections parallel to the surface. The sections were collected and pooled according to the following scheme: # 1 = incomplete cuts; # 2–5 = 2 × 25 μm sections/pool; # 6–9 = 4 × 25 μm sections/pool; # 10 + 11 = 8 × 25 μm sections/pool; # 12 = residual tissue. The thickness of the incomplete sections and the remaining skin was calculated with the help of the weights of the sections related to a standard section with known weight and thickness.

2.2.4. Assay method and HPLC-procedure

The flufenamic acid was extracted from the adhesive tape, the skin sections and the filter paper with 1.5 ml 0.05 N NaOH. For a detailed description of the extraction procedure and the HPLC analysis (detection limit 20 ng/ml) see Wagner et al. [2]. The linearity of the HPLC method was verified over a range from 50 to 10,000 ng/ml (linearity $r >= 0.999$ and accuracy 97.8 ± 3.8%), and the repeatability was determined to be ± 1.83%.

2.3. Characterisation of the semisolid formulations

- *Saturating concentration of the drug in the solvents of the hydrogels* (see Table 1). 0.5 g flufenamic acid was added to 5.0 ml of the investigated liquid phase of the formulations and was mixed with a magnetic stirring bar at room temperature for 17.5 h. Samples of the solution were centrifuged at 10286 × *g* for 10 min and subsequently filtered through a teflon filter with 0.2 μm pore size, diluted with 0.05 N NaOH 1:50, and their drug contents were measured by HPLC.
- *Drug dispersion*. The drug dispersion was microscopically inspected using an Olympus microscope BH10 (magnification 93.75-fold).
- *Bleeding number of the wool alcohol ointments*. The lipophilic ointment bases were placed in penetrometer bowls and stored for 1 h at 32 °C. After smoothing the surface of the ointment, the bowl was set upside-down on a filter paper type 595 (Schleicher and Schuell, Dassel, D) and stored for 6 h at 32 °C. The diameter of the bleeding zone was determined thereafter.
- *Spreading*. 0.5 g of the drug formulations were placed between 2 glass plates and a weight of 2.5 kg was put on the top of the arrangement for 5 min. After removing the weight, the areas of the drug formulations between the glass plates were determined.
- *Drug liberation*. The drug liberation experiments were carried out by analogy to the experiments with the FD-C, but using a dialysis membrane (Dianorm GmbH, Muenchen, D; cut off 10,000) instead of human skin. Samples were drawn at regular time intervals, replacing the removed acceptor medium with fresh buffer. In all cases sink conditions were maintained.

3. Results and discussion

3.1. Physico-chemical characteristics of the drug formulations

The drug formulations differed with respect to their hydrophilic and lipophilic properties and regarding the dispersion of the drug within the formulation (Table 1). Due to the fact that only dissolved drug is able to penetrate from an applied drug formulation into the skin, the concentration of saturation of flufenamic acid in the different phases of the semisolid preparations was determined. Within the polyacrylate gels isopropanol increased the solubility of flufenamic acid around tenfold, while propylene glycol reduced the solubility of flufenamic acid in the liquid phase to a half (each in comparison to the aqueous phase).

Lipophilic ointment bases lose part of their liquid components after some time of storage, which is known as bleeding. This bleeding, caused by syneresis, is associated with a hardening of the gel's structure. As reported by Loth [8], the liquid components are able to penetrate into the SC, and therefore might influence or disrupt its barrier properties. As demonstrated in Table 1, the bleeding number was not influenced by the incorporation of the different drug amounts. However, the addition of 50% water (AWAO) resulted in a reduction of the bleeding effect caused by a decrease of the lipophilic liquid components. In addition, it might be speculated that some of the liquid excipients of

WAO were bound to the emulsion structure, and therefore their bleeding was lower, too.

At the beginning of the penetration of drugs from an ointment layer into the skin (= invasion of the drug), the liberation of the drug out of the ointment might be rate determining. For this reason, liberation experiments were carried out using a hydrophilic dialysis membrane to separate the semisolid preparation physically from the liquid receptor compartment. From literature it is known that the buffer medium might diffuse through the membrane and interact especially with the hydrophilic semisolid bases [9,10]. However, visual inspections of the preparations at the end of the experiments gave no hint of an invasion of acceptor fluid into the preparation.

Regardless of the character of the ointment base, all formulations showed typical liberation profiles (Fig. 1). In all cases Higuchi square-root-of-time plots [11] gave linear profiles (Fig. 1) with correlation coefficients of $r = 0.975–0.985$ (lipophilic vehicles) and $r = 0.989–0.998$ (hydrophilic vehicles). These results demonstrate that the release of the applied semisolid preparation is the rate limiting step for the drug liberation and that it was not controlled by the artificial membrane. The detected rank order of drug liberation can be stated as follows: lipophilic

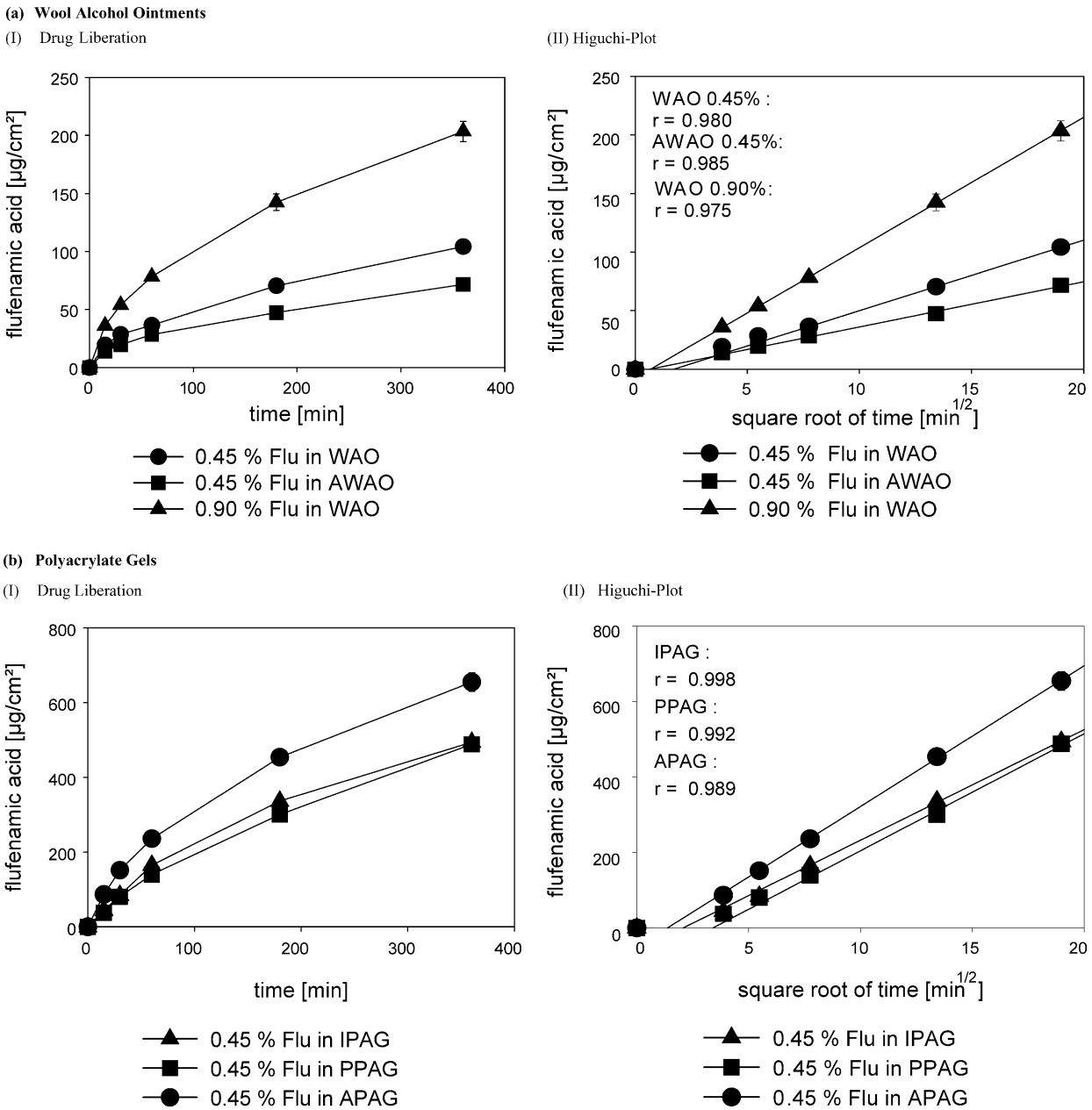

Fig. 1. Drug liberation from varying vehicles through a dialysis membrane (µg/cm^2 ± SE; $n = 3$). (a) Wool alcohol ointments, (b) polyacrylate gels.

ointment bases—WAO (0.90%) > WAO(0.45%) > AWAO (0,45%); hydrophilic ointment bases—APAG > IPAG = PPAG.

For the lipophilic drug formulations the influence of the drug concentration within the vehicles on the release rate is shown in Fig. 1a (increase of the liberation around twofold). However, the incorporation of water slightly reduced the liberated drug amounts for the same drug concentration. This might be explained first by the higher tortuosity of the lipoidal phase within the cream system, which caused longer diffusion pathways. Second, the partitioning of flufenamic acid into the aqueous phase reduced the drug content in the lipoidal phase. Third, the slightly increased viscosity of the formulation (Table 1) reduced the diffusion coefficient. In comparison with the lipophilic formulations, the hydrophilic ointment bases generally led to an increase of drug release (Fig. 1b). A reduced but dissimilar viscosity (Table 1) and the presence of varying amounts of dissolved and suspended drug (depending on the concentration of saturation within the gel systems), respectively, might be responsible for the observed effects (diffusion coefficient increased, highest possible thermodynamic activity [12]).

3.2. Drug amounts in the stratum corneum and the deeper skin layers

Table 2 illustrates the drug amounts present in the SC and the DSL after three different incubation periods and the application of the six drug formulations in two in vitro test systems.

At the end of the experiments no drug could be detected either in the phosphate buffer used as acceptor medium in the FD-C, or in the filter paper lying beneath the skin in the SB-M. Thus, for all experiments sink conditions could be expected for the DSL.

3.2.1. Liphophilic ointment bases

Considering the data for the *SC*, the differences between the two in vitro test systems were small (Table 2a). In general, the penetrated drug amounts reached a plateau after one hour of incubation whereas the increase in the drug amounts up to this level occurred with varying speed. The absolute drug amounts in the SC were more or less identical using the 0.45% flufenamic-acid-containing formulations, and around twice as high with the 0.90% flufenamic-acid-containing ointment base.

In the *DSL* (Table 2b), rising drug amounts with increasing incubation times were detected for both in vitro test systems and all formulations. Comparing the water-free preparations WAO 0.45% and WAO 0.90% in the SB-M, a constant increase in the drug amounts could be observed over the experimental time period, whereas with the FD-C a steep increase occurred after 1 hour. This is in accordance with previous results [2], where for the FD-C a counter current of receptor fluid was expected during the first hour of the incubation period. Concerning the AWAO 0.45% the drug amounts were rising constantly in both test models. The reason might be that due to the co-administration of water from the donor side, water penetrated in the same direction as the drug which might reduce the effects of the counter current of the receptor fluid in the FD-C.

The results of the penetration experiments with 0.45% flufenamic acid in WAO and AWAO differed from each other, especially after 180 min. Here, the water incorporated in the AWAO influenced the drug's penetration with longer incubation times. Lippold [13] found comparable results using w/o-emulsions and explained his data on the basis of increased hydration of the skin. Obviously, the hydration state of the SC was more decisive for the drug penetration than the lipophilic liquid compounds of the ointment bases and their tendency to diffuse into the SC, where they could

Table 2
Drug amounts (ng/cm^2) penetrated into the stratum corneum and the deeper skin layers after the use of two different in vitro test systems (mean ± SE; $n = 2–4$)

Form	SB-M (ng/cm^2 ± SE)			FD-C (ng/cm^2 ± SE)		
	30 min	60 min	180 min	30 min	60 min	180 min
(a) Stratum corneum						
WAO; 0.45%	2165.1 ± 644.4	1989.8 ± 41.0	1906.8 ± 280.1	1080.4 ± 146.0	1842.2 ± 41.8	1842.0 ± 259.0
AWAO; 0.45%	1841.1 ± 49.0	2370.8 ± 1.2	2154.1 ± 103.45	1639.7 ± 124.8	2049.7 ± 412.8	2157.8 ± 449.7
WAO; 0.90%	4115.2 ± 88.7	4331.1 ± 208.7	4837.9 ± 413.7	2779.3 ± 197.8	5251.0 ± 347.7	5233.6 ± 493.8
APAG; 0.45%	3656.2 ± 398.1	5659.6 ± 1047.5	5102.7 ± 890.5	3531.4 ± 45.0	3760.6 ± 253.0	4057.8 ± 445.7
PPAG; 0.45%	2743.3 ± 513.1	4406.7 ± 1185.1	3554.0 ± 59.9	2304.4 ± 113.4	2149.3 ± 180.4	2475.1 ± 341.2
IPAG; 0.45%	11817.5 ± 947.5	9577.0 ± 551.5	12586.1 ± 349.4	10474.9 ± 240.0	9228.2 ± 1517.0	11156.2 ± 622.4
(b) Deeper skin layers						
WAO; 0.45%	536.5 ± 1.9	1862.15 ± 708.7	6786.6 ± 724.6	104.8 ± 10.6	488.0 ± 152.9	3726.3 ± 1777.0
AWAO; 0.45%	218.1 ± 64.5	1699.3 ± 416.3	9119.6 ± 466.0	621.8 ± 521.8	1653.0 ± 461.8	7054.7 ± 199.8
WAO; 0.90%	786.9 ± 35.7	2460.8 ± 346.8	10856.9 ± 1640.5	542.0 ± 393.1	750.3 ± 244.7	10006.8 ± 2240.6
APAG; 0.45%	3005.7 ± 456.8	4845.1 ± 81.2	19210.2 ± 950.8	2137.8 ± 143.9	3234.0 ± 728.0	15624.9 ± 2835.4
PPAG; 0.45%	998.1 ± 141.3	3342.0 ± 166.8	13829.5 ± 351.0	306.9 ± 31.1	1492.8 ± 26.6	7359.6 ± 174.3
IPAG; 0.45%	11925.5 ± 2846.1	19340.0 ± 29.9	61421.7 ± 6569.8	7190.4 ± 663.8	14981.8 ± 4478.3	47374.5 ± 15277.8

interact with the SC lipids so as to change barrier properties [14]. This seemed astonishing, because flufenamic acid is a lipophilic drug. Loth [8] distinguished between two mechanisms where hydrophilic and lipophilic liquid compounds could interact with the SC. While the water molecules hydrate the polar head groups of the lipids, the lipophilic liquid components migrate into the intercellular lipid layers, disturb their order of packing and possibly liquefy the intercellular material. The result is a decrease in the diffusion resistance of the SC.

3.2.2. Hydrophilic ointment bases

An analogous test series was carried out with hydrophilic ointment bases (Table 2). With this set of experiments it was possible to examine the influence of two widely used penetration enhancers, isopropanol and propylene glycol. In contrast to the investigations with the lipophilic ointment bases, the drug amounts within the gels were kept constant at 0.45% flufenamic acid.

In accordance with the data obtained for the lipophilic ointment bases, the results of the penetration experiments differed only slightly within the *SC* in dependence on the model. However, the absolute drug amounts obtained with the SB-M exceeded the amounts obtained with the FD-C. With rising incubation times a constant drug amount was established in the SC which seemed to take longer time periods with APAG and PPAG than with IPAG. After applying IPAG the SC was saturated with drug within 30 min. For the detected drug amounts in the SC the following rank order could be detected: IPAG > APAG > PPAG. These findings were in accordance with the concentration of saturation measured for each liquid phase of the gels. However, on the basis of the liberation experiments carried out with all formulations one would have assumed that APAG would deliver the drug faster to the skin than IPAG. Thus, other effects of the alcohols and interactions with the skin had to be taken into account.

Water as well as propylene glycol and isopropanol are described in literature as penetration enhancers which are defined as substances without any therapeutic effect by themselves. However, they have the ability to diffuse into and distribute themselves within the SC, to interact with the SC's compounds and finally to reduce the diffusion resistance of the skin. Besides, some penetration enhancers are able to increase the thermodynamic activity of drugs, which leads to a higher drug flux [15]. The effect of water on the barrier function of the SC has already been discussed in the previous paragraph titled 'lipophilic ointment bases'.

The action of propylene glycol and isopropanol as penetration enhancers can be stated as follows. Propylene glycol is able to increase or decrease the penetration of drugs. According to Pandey et al. [16] it influences the microviscosity of the vehicle. Mollgaard and Hoelgaard [17] proposed that a concentration of propylene glycol in which the drug concentration is saturating is optimal. The effect of this excipient depends on its concentration in the vehicle and varies in dependence on the drug [18]. Mayer et al. [19] describe propylene glycol as a semi-polar solvent which is able to penetrate into the epidermis carrying dissolved drug. Other labs interpret this 'solvent drag'-theory as propylene glycol acting as a solubilizer. In this view, the effect of propylene glycol is analogous to the effect of water in that both agents are thought to hydrogen bond to the polar head groups of the lipids in the SC. Again this leads to an enlargement of the distance between the lipid molecules in combination with a lateral extension of the alkyl chains [20,21]. Besides, a modification is described with the shift of the solubility parameters of the skin in the direction of the drug substance. The solubility of the drug in the outermost layers of the skin will be increased and this, in turn, improves the flux [22]. As an additional effect of propylene glycol, the dehydration of the corneocytes might be relevant [20,23], but need not to lead to a dehydration of the SC as a consequence. A change of the water binding capacity is also possible resulting in reduced barrier properties of the SC and increased drug penetration. In our study the addition of propylene glycol to an aqueous polyacrylate gel clearly led to decreased penetration rather than penetration enhancement for the lipophilic drug flufenamic acid.

On the other hand, the influence of isopropanol was evident. Comparing IPAG to APAG a 2–3 fold enhancement was detected. Like propylene glycol, isopronanol is also able to penetrate into the lipid layers of the SC, which leads to a change of the solubility of the drug within these regions. Additionally, isopropanol can extract lipids and proteins from the SC, too, and thus may increase the porosity of the SC resulting in facilitated drug diffusion [20–22]. These effects are independent of the drug used.

In the *DSL*, increasing drug amounts were detected in a time dependent manner, no matter which vehicle was applied on the skin. However, in comparison to the lipophilic ointments higher drug amounts were reached. The reason for these results might lead back to the hydration state of the skin which was increased by water from the vehicle and co-penetration of propylene glycol and isopropanol, respectively. These effects are more pronounced in the SB-M. In correspondence with the results obtained with the lipophilic ointment bases, the drug amounts determined for the SB-M experiments were higher than the drug amounts gained with the FD-C. The differing concentration gradients between the SC and the DSL, which were steeper for the SB-M than the FD-C might be a reason for these findings. IPAG demonstrated drug amounts threefold higher than APAG and PPAG, which could be caused by the co-entering of isopropanol in the DSL resulting in an increase of drug solubility in these skin layers.

Regardless of the system used all vehicles led to a high hydration state of the skin caused by the water present in each drug formulation. Furthermore, all experiments were carried out using an infinite dose under occlusive

conditions, thus significant water loss during the investigation can be ruled out. Therefore, a completely hydrated SC in combination with reduced barrier properties of the SC was present in both models, and consequently, in contrast to the lipophilic bases, the effect of the liquid receptor phase (phosphate buffer, pH 7.4) in the FD-C was levelled out.

3.3. Comparison of lipophilic and hydrophilic vehicles

Besides a qualitative comparison between the vehicles tested and an attempt to explain the data, the vehicles were also compared quantitatively. For this investigation, 0.45% flufenamic acid in WAO was used as a standard. The following ratios could be calculated after 3 hours of incubation (based on the data given in Table 2):

- in the SC:

SB-M: lipohpilic: 1.1-fold (AWAO), 2.5-fold (WAO, 0.9%)
hydrophilic: 1.9-fold (PPAG), 2.7-fold (APAG), and 6.6-fold (IPAG)
FD-C: lipophilic 1.2-fold (AWAO), 2.8-fold (WAO, 0.9%)
hydrophilic: 1.3-fold (PPAG), 2.2-fold (APAG), and 6.1-fold (IPAG)

Although the extent of the increase was not exactly the same in both in vitro test systems, the same rank order was obtained. Finally, the application of hydrophilic vehicles led to an increased drug penetration, which could not be achieved with lipophilic ointment bases, and a doubling of the drug concentration.

- in the DSL:

SB-M: lipophilic: 1.3-fold (AWAO), 1.6-fold (WAO, 0.9%)
hydrophilic: 2.0-fold (PPAG), 2.8-fold (APAG) and 9.0-fold (IPAG)
FD-C: lipophilic: 1.9-fold (AWAO), 2.7-fold (WAO, 0.9%)
hydrophilic: 2.0-fold (PPAG), 4.2-fold (APAG) and 12.7-fold (IPAG)

In accordance with the results obtained in the SC, the ratios between both in vitro test systems and between the SC and the DSL were not exactly the same. Nevertheless the same trend could be observed.

Based on the present experimental design, it could be expected that a steady-state would be established in the SC with rising incubation times. The detection of a plateau indicated the fact that quasi steady-state conditions might be expected. Based on Michaelis–Menten-kinetics, an Eq. (1) was established in previous studies [2] which allowed the calculation of the steady-state drug amount present in the SC and the time after which a 99% drug amount is reached.

Table 3
Mean values for m_{ss}, $t_{ss/2}$, $t_{m99\%}$ calculated with Eq. (1)

		m_{ss} (ng/cm^2 ± SE)	$t_{ss/2}$ (h ± SE)	$t_{m99\%}$ (h)	r
SB-M	WAO-0.45%	2061.77 ± 230.31	0.12 ± 0.13	11.88	0.962
	AWAO-0.45%	2356.20 ± 172.26	0.10 ± 0.07	9.90	0.977
	WAO-0.90%	4947.29 ± 277.60	0.11 ± 0.05	10.89	0.986
	APAG-0.45%	5878.44 ± 1097.11	0.21 ± 0.22	20.79	0.883
	PPAG-0.45%	4165.15 ± 962.40	0.16 ± 0.25	15.84	0.827
	IPAG-0.45%	11661.36 ± 1258.75	0.03 ± 0.09	2.97	0.949
FD-C	WAO-0.45%	2188.11 ± 322.10	0.38 ± 0.22	37.62	0.935
	AWAO-0.45%	2342.05 ± 386.98	0.19 ± 0.19	18.81	0.903
	WAO-0.90%	6383.62 ± 976.57	0.45 ± 0.24	44.55	0.935
	APAG-0.45%	4157.32 ± 283.75	0.09 ± 0.06	8.91	0.979
	PPAG-0.45%	2411.17 ± 233.91	0.04 ± 0.08	3.96	0.958
	IPAG-0.45%	11244.30 ± 228.93	0.04 ± 0.01	3.96	0.999

$$m_{act} = \frac{m_{ss} \times t_{inc}}{t_{ss/2} + t_{inc}} \quad (1)$$

where m_{ss}, quasi steady-state drug amount in the SC; $t_{ss/2}$, incubation time after which 1/2 m_{ss} is reached; t_{inc}, incubation time; and m_{act}, actual drug amounts at time t_{inc}.

According to the calculated data in Table 3, this equation was valid for both in vitro test systems, lipophilic and hydrophilic vehicles and dissolved as well as suspended drug. While the differences of the $t_{ss/2}$-values were only small with relatively high standard variations, the m_{ss}-values led to results which were in accordance with the ratios given above. Therefore, the question arose as to whether it would be possible to correlate these m_{ss}-values of the SB-M and the FD-C with one another. The results of this correlation are given in Fig. 2. The correlation coefficient clearly indicates that a linear relation between both data sets exists. The absolute values measured with each test system were different, but the ratios were the same. This fact was supported by the value of the slope of the regression line, which practically equalled '1' (exact value: 0.954 ± 0.172).

Assuming that the drug amounts present in the SC were lower than the m_{ss}-values and sink conditions existed in the DSL, a relationship between the drug amount in the SC

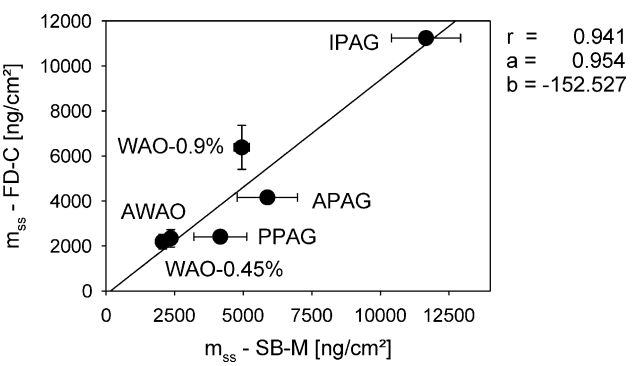

Fig. 2. Correlation of the m_{ss}-values of the Saarbruecken penetration model with the m_{ss}-values of the Franz Diffusion Cell (mean ± SE).

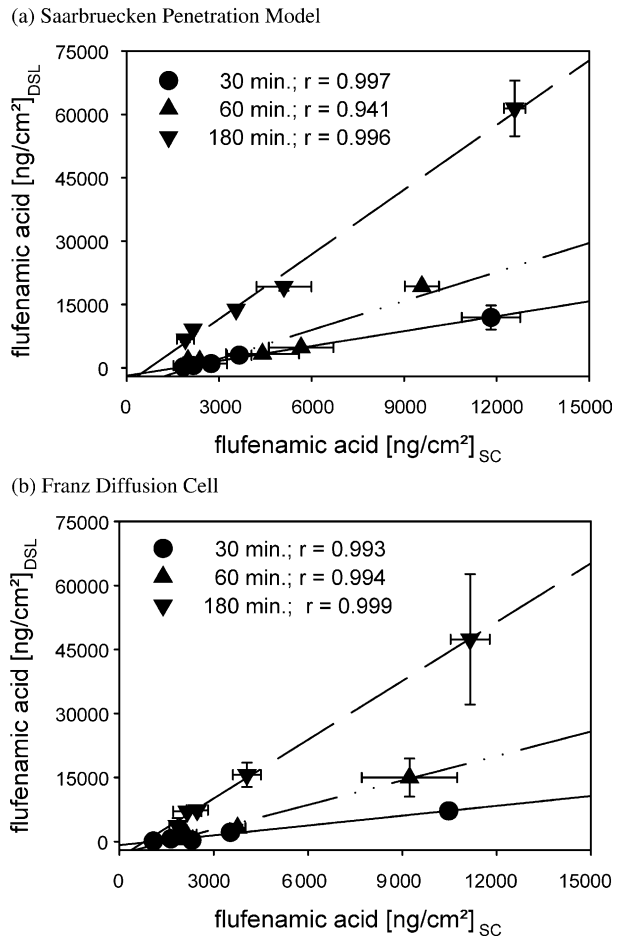

Fig. 3. Correlation between stratum corneum data and values obtained in the deeper skin layers (mean ± SE).

and the DSL should exist. Theobald [24] reported an empirical relation between the drug amounts in the SC and the DSL for the drug hydrocortisone using incubation times of 1 and 3 h and four different vehicles. However, no information about the concentration of saturation in the SC and sink conditions were given. As indicated by the $t_{m99\%}$-values calculated according to Eq. (1) (Table 3), steady-state conditions in the SC could not be assumed for our experiments up to 3 h. In addition, sink conditions could also be assumed, as no drug was detected either in the filter paper beneath the skin in the SB-M or in the liquid receptor fluid in the FD-C. The results achieved for flufenamic acid are illustrated in Fig. 3. The correlation coefficients indicated that, no matter which in vitro test system was used, a linear relationship between the drug amounts penetrated into the SC and the drug amounts present in the DSL could be found for incubation times between 30 and 180 min.

4. Summary and conclusions

In the present work the influence of different hydrophilic and lipophilic vehicles (hydrogels and ointments) and dissolved and suspended drug using two in vitro test systems (SB-M and FD-C) was investigated. The effects of drug concentrations and different excipients within the formulations were examined and explanations were given for the varying results.

Although the absolute values achieved with both test systems were different, possibilities are demonstrated of directly comparing both sets of data concerning the SC using the quasi steady-state drug amount in the SC. This relationship might provide a means to compare already available data obtained in a variety of labs with different in vitro models and thus to reduce the experimental work in the future.

Besides, an attempt was made to find a relationship between SC data and values of the DSL. A direct linear correlation between both sets of data was observed, as long as the quasi steady-state drug amounts in the SC were not reached. An in vivo/in vitro-correlation has already been established for the SC [2] and the DSL [25]. Therefore, this result provides a means to predict in vivo data for the DSL from in vitro data (SC + DSL) combined with in vivo data (only SC). Hence, non-invasive methods would be sufficient under in vivo conditions (tape stripping technique). This would reduce risks to the volunteers, reduce the costs and simplify the development of new drug formulations. However, a verification of these data with other drugs is a worthwhile goal!

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