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# Dermal peptide delivery using enhancer molecules and colloidal carrier systems – part V[#]: Influence of enhancers on the permeation of PKEK through snake skin

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In a previous study, it was shown, that shed snake skin is a good alternative model membrane for the human stratum corneum (SC). In this study, the influence of the enhancers dimethyl sulfoxide (DMSO), 1,2-propanediol, 1,3-butanediol, 1,2-pentanediol, 1,2-hexanediol and 1,2-octanediol in a concentration of 10 % on the permeation of l-prolyl- l-lysyl-l-α-glutamyl-l-lysin (PKEK) through shed snake skin was conducted. Pharmacokinetic parameters (diffusion coefficient, permeation coefficient, t-lag, Flux) were calculated. All examinations were performed on the skin of an individual and thus allowed a very good comparability of the data. All enhancers have overcome the shed snake skin and could be proven in the acceptor. DMSO does not affect the permeability of the membrane. Nevertheless, PKEK permeates faster in the presence of DMSO than PKEK being used alone. PKEK permeated the same, no matter if an auxiliary material was added or not. Without their addition, in all other enhancers no significant difference towards permeation could be determined.

## 1. Introduction

Influencing the skin barrier is an important topic. There are several ways to improve the penetration of pharmaceutical substances. The active substance itself can be modified or a suitable vehicle can be chosen such as nanoparticles, liposomes or microemulsions. Through enhancer or hydration, the SC can be changed or bypassed through microneedles. Even through physical methods such as iontophoresis or electroporation, the penetration can be increased. Also a combination of these methods is possible (Barry 2001; Daniels 2004; Fernando et al. 2012; Raphael et al. 2013). The focus of this study was on improving penetration using various enhancers. Ideally, enhancers should promote the penetration of molecules without causing allergies or skin irritation and should only change the skin barrier temporarily for active substances to enter the body without the loss of endogenous substances. Furthermore, they should not have pharmacological activity and for instance, should not bind to the receptors. They should be compatible with possible auxiliary materials and pharmaceutical substances and leave pleasant feeling on the skin. Until now, no substance has been discovered which possesses all these ideal properties (Williams and Barry 2012). How exactly enhancers affect the skin barrier has not been conclusively researched. However, various causes are discussed. Among other things it has been assumed that the lamellar arrangement of lipids in the SC is disturbed and can result in a phase separation. This phase separation is initiated by either inverse micelles in the area of the lipophilic chains or through water molecules in the region of the polar heads (Barry 2001, 2004; Engelbrecht et al. 2011, 2012; Williams and Barry 2012). They can also increase the solubility in the SC, change skin proteins (keratin), interact with the desmosomes, elute lipids or destroy the water structure (Aungst et al. 1986; Barry 2001, 2004). In the following, the enhancers used in this study are briefly described.

DMSO is one of the first and well studied enhancers which promotes very fast the penetration of both hydrophilic and lipophilic substances (Coldman et al. 1971; Maibach and Feldmann 1967; Myoung and Choi 2002; Williams and Barry 2012). The disadvantage is that for an optimum effect, concentrations of at least 60 % are necessary, which may cause erythema (Anigbogu et al. 1995; Coldman et al. 1971; Kligman 1965; Notman et al. 2008). DMSO promotes the penetration of substances by various mechanisms: it dissolves lipids thus forming water-filled pores (Allenby et al. 1969). Through DMSO, the keratin conformation in the cell changes from an α-helix to the more space-taking β-pleated sheet (Anigbogu et al. 1995; Brezesinski et al. 2008; Oertel 1977). In addition, it interacts with the head groups of some lipids of the bilayer and thus disturbs their order, since the solvation shell of the DMSO molecules is larger than that of the displaced water molecules (Barry 1987; Guillard et al. 2009). At the same time, DMSO present in the SC promotes the diffusion of the active substance from the vehicle into the skin (Williams and Barry 2012).

Also, fatty alcohols respectively alkanols have properties which promote penetration. It has been shown, for example, that 1-butanol was the most effective enhancer of levonorgestrel penetration through rat skin (Friend et al. 1988). They also lead to a slight change in the lipid order within the bilayer in the SC (Brinkmann and Müller-Goymann 2005; Williams and Barry 2012). For 1,2-propanediol the so-called "solvent drag effect" is described as follows: It is able to take other substances into the tissue and is often used as a co-solvent (Hoelgaard and Møllgaard 1985). It has been shown that the depth of penetration of 1,2-propanediol correlates with the depth of penetration of the co-administered substance (Pudney et al. 2007). 1,2-Propanediol settles into the lipid head groups thus increasing the solubility of the active substance. Through this, the driving force of the diffusion changes and the absorption of the active substance into the skin is eased (Kasting et al. 1993; Watkinson et al. 2009; Williams and Barry 2012). Likewise, it has been described for 1,2-pentanediol properties which promote penetration (Duracher et al. 2009; Heuschkel et al. 2008). Because of its antimicrobial and preservative properties, this enhancer is particularly interesting for the use in dermatological formulations (Pillai et al. 2005).

PKEK is a tetrapeptide which is used in cosmetics and is commercially available under the name TEGO®Pep 4-Even. It inhibits the pigmentation process and is said to have a whitening effect on aging skin. It has already been shown that PKEK reduces the UVB-induced expression of interleukins, TNF-α and proopiomelanocorticotropin (POMC). PKEK has a high molecular weight of 500.59 g /mol and shows many ionizable groups thus it is very hydrophilic at a logP of -1.599 ± 0.909. With 8 proton donors and 10 acceptors, PKEK has great potential for interaction (Neubert et al. 2018).

In the previous paper "Dermal Peptide Delivery Using Enhancer Molecules and Colloidal Carrier Systems – Part IV[#]: Search for an alternative model membrane for future ATR permeation studies using PKEK as model substance" it has been shown that shed snake skin is an alternative model membrane to the human SC.

Therefore, shed snake skin was used in this study to investigate the influence of the enhancers DMSO, 1,2-propanediol, 1,3-butanediol, 1,2-pentanediol, 1,2-hexanediol and 1,2-octanediol.

## 2. Investigations and results

The evaluation was carried out analogously to that one described earlier ("Dermal Peptide Delivery Using Enhancer Molecules and Colloidal Carrier Systems – Part IV[#]: Search for an alternative model membrane for future ATR permeation studies using PKEK as model substance"). Figs. 1 and 2 show exemplarily the concentration time course of PKEK in the presence of 10 % DMSO.

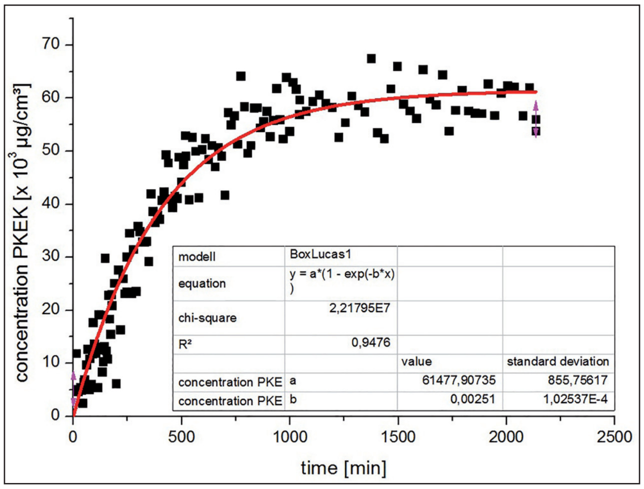

Fig. 1: Concentration time course of PKEK in the acceptor in the presence of 10 % DMSO in deuterium oxide ($D_2O$). The red line is the Box-Lucas-Plot according to the formula $\gamma = a \cdot (1 - e^{-bx})$

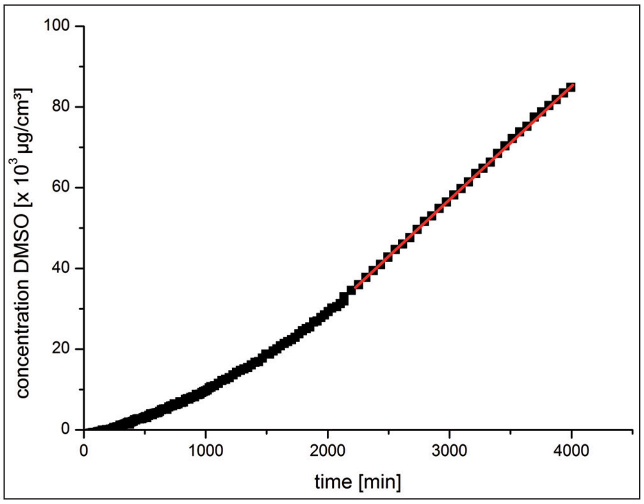

Fig. 2: Concentration time course of DMSO in the acceptor in the presence of PKEK in $D_2O$. The red line is the linear fit.

Depending on how fast the steady state of the skin adjusts, at first an exponential increase in the concentration within the acceptor was seen (e.g. in this case with DMSO). When the steady state within the skin was reached, the graph went into a linear course. At PKEK, the steady state was so fast that no exponential increase was perceived. Basically, the curve progression for PKEK was the same, regardless of the auxiliary material itself.

Through a linear fit in each linear region of the concentration-time course, the increase was obtained, which is a measure of the mass flow or Flux. The Flux was used to determine to what extent the auxiliary materials influenced the permeation of PKEK. It could also be derived whether and how fast the auxiliary materials permeated themselves.

It could be seen that PKEK had quickly reached its maximum of concentration in the acceptor. In the presence of DMSO a Flux of 66.55±11.63 µg/cm$^2$min$^{-1}$ resulted.

DMSO required more time to reach the steady state in the skin and had a lower Flux of 26.28±3.03 µg/cm$^2$min$^{-1}$. At the end of the experiment, the maximum concentration was not even reached. Nevertheless, it should be noted that DMSO has also overcome the SC.

The permeation coefficient was calculated from the Flux. For PKEK, a permeation coefficient of 6.65±1.16 × 10$^{-4}$ cm/min and for DMSO 2.63 ± 0.30 × 10$^{-4}$ cm/min was obtained.

Generally, it was noticeable that the individual measuring points of PKEK fluctuated more than those of DMSO. This was because the two DMSO peaks were, in relation to the PKEK, quite larger. Resulting from the inaccuracy of the device, the larger the signal and with it the area, the lower the measurement variations. This could be compensated through a higher PKEK concentration since the peak area is concentration dependent. However, an increase of PKEK concentrations in the donor at the current experiments would lead to an unreasonable amount of costs.

This also applies to the other auxiliary materials. With sufficient signal intensity and lower cost of materials, a compromise has been reached.

Figure 3 shows the concentration time courses of the other auxiliary materials. Their evaluation was determined analogously.

There are several ways how substances could overcome the skin barrier. The intercellular route is the preferred diffusion path. Thereby diffusion of lipophilic substances along the hydrocarbon chains of the lamellar lipid matrix and diffusion of hydrophilic substances along the hydrated head groups occur. Lipophilic substances overcome the skin barrier better than hydrophilic substances whereas lipophilic substances often accumulate in the stratum corneum (Barry 1987, Barry 2001; Neubert and Wepf 2007). It appears that with increasing chain length of the alkanol the auxiliary material reaches the donor faster. With the increasing chain length, the molecule becomes more lipophilic and can overcome the stratum corneum more easily. Although the molecular weight also increases with the chain length but still, 1,2-octanediol is less than 500 Da. Molecules, which are larger than 500 Da can hardly overcome the skin barrier. Thus it seems, that more optimal ratio of lipophilicity, size, and interaction potential with the SC exists at 1,2-hexanediol

**Table 1: Comparison of the permeation parameters [n = 3] of a 10% PKEK solution in $D_2O$ through shed snake skin in the presence of the respective auxiliary material with a concentration of 10%. In each case given is the arithmetic mean ± standard deviation.**

| Auxiliary material | D [PKEK] in x10$^{-9}$ cm²/min | $t_{lag}$ [PKEK] in min | J [PKEK] in µg/cm²min$^{-1}$ | P [PKEK] in x10$^{-4}$ cm/min |
|---|---|---|---|---|
| **1,2-propanediol** | 5.06 ± 0.99 | 195 ± 36 | 63.15 ± 10.52 | 6.31 ± 1.05 |
| **1,3-butanediol** | 4.76 ± 1.48 | 215 ± 63 | 61.44 ± 4.32 | 6.14 ± 0.77 |
| **1,2-pentanediol** | 5.41 ± 1.68 | 188 ± 50 | 75.10 ± 38.82 | 7.51 ± 3.88 |
| **1,2-hexanediol** | 5.63 ± 0.70 | 173 ± 20 | 45.74 ± 8.41 | 4.57 ± 0.84 |
| **1,2-octanediol** | 6.36 ± 0.35 | 152 ± 8 | 61.12 ± 15.62 | 6.11 ± 1.56 |
| **DMSO** | 9.05 ± 0.76 | 107 ± 9 | 66.55 ± 11.63 | 6.65 ± 1.16 |
| **without auxiliary materials** | 7.03 ± 1.32 | 124 ± 38 | 59.56 ± 10.37 | 5.96 ± 1.04 |

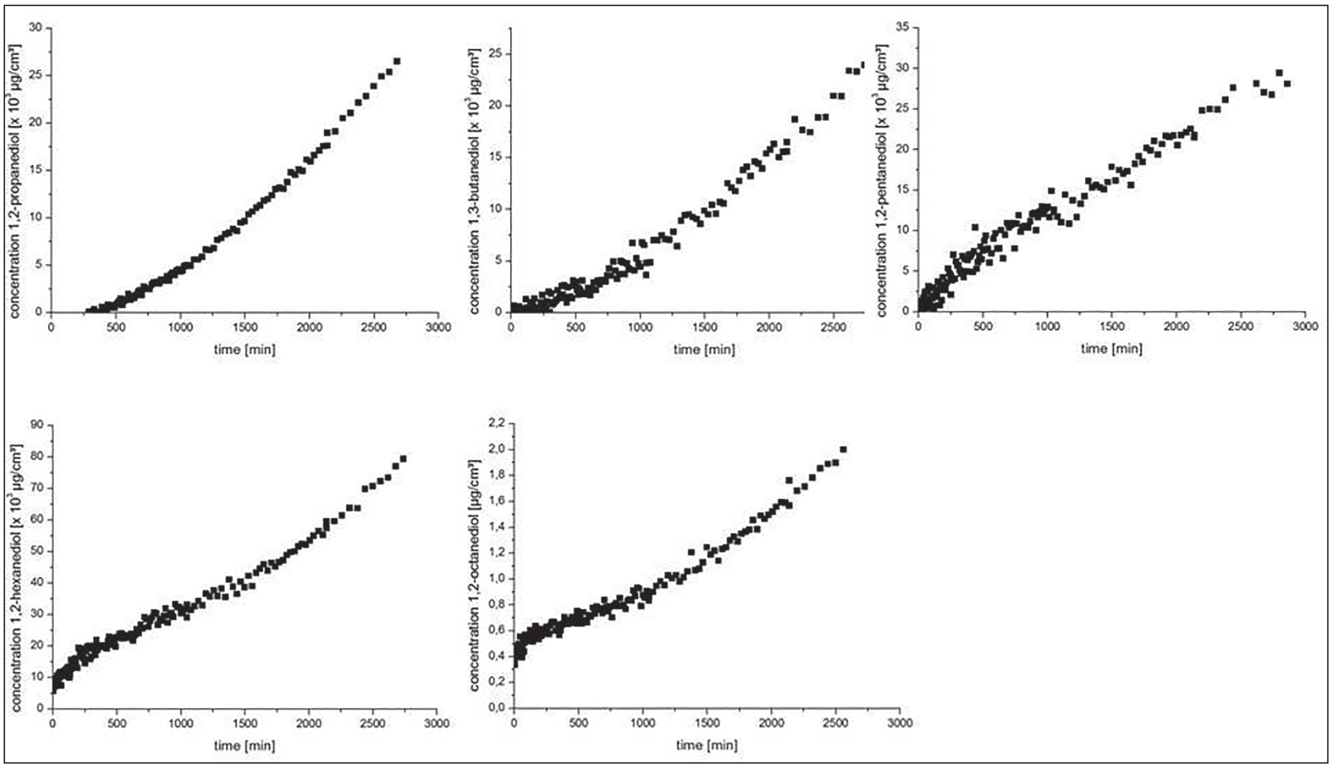

Fig. 3: Concentration time courses of the various auxiliary materials within the acceptor. Permeation was done by shed snake skin in the presence of 10% PKEK in $D_2O$. Each auxiliary material had a concentration of 10 %. 1,2-propanediol at the top left, 1,3-butanediol at the top center, 1,2-pentanediol at the top right, lower left 1,2-hexanediol and lower middle 1,2-octanediol.

and 1,2-octanediol, than at the shorter alkanols. This explains why the t-lag of 1,2-propanediol is the largest of all the diols tested and why 1,2-hexanediol and 1,2-octanediol begin to show a burst effect.

It has been proven, that all auxiliary materials - DMSO, 1,2-propanediol, 1,3-butanediol, 1,2-pentanediol, 1,2-hexanediol, and 1,2-octanediol – had overcome the snake skin and permeated within the acceptor. Therefore it can be assumed that also the auxiliary materials can overcome the human SC.

Table 1 summarizes the results of PKEK permeation in presence of various auxiliary materials. It can be seen that PKEK permeated at an almost similar speed in the presence of all auxiliary materials, since the permeation coefficient and the Flux were of a similar order of magnitude. In this experiment, the diffusion coefficient was of interest, since the auxiliary material as part of the solvent $D_2O$ could influence the properties of the 10% PKEK solution.

Whether the differences were statistically significant, was analogically analysed in our previous study “Dermal Peptide Delivery Using Enhancer Molecules and Colloidal Carrier Systems – Part IV: Search for an alternative model membrane for future ATR permeation studies using PKEK as model substance”, about the investigation of the different isolated SC through the geometrical mean and their upper and lower limits. This is shown in the following Tables 2, 3 and 4.

Statistically, there was no significant difference in the PKEK diffusion coefficient between the polyhydric alkanols 1,2-propanediol, 1,3-butanediol, 1,2-pentanediol, 1,2-hexanediol, and 1,2-octanediol, meaning these auxiliary materials influenced the permeation of PKEK to the same extent.

However, DMSO as an auxiliary material, had a statistically significant difference from the polyvalent alkanols, to be more specific, had a significantly larger diffusion coefficient than the polyhydric alkanols. In other words, with DMSO serving as an additive, PKEK diffused faster into and through the skin. As a result, the concentration of PKEK in the acceptor increased faster than with the addition of a polyhydric alkanol. Whether there is a significant difference between permeation without enhancer or with a polyvalent alkanol enhancer was not certain. This would require a larger number of experiments.

As can be seen in Table 3, there was no statistically significant difference between the permeation coefficients of PKEK in the presence of the various auxiliary materials compared to the permeation test without the addition of auxiliary material. This

**Table 2: The geometric mean values of the diffusion coefficient of PKEK in the presence of the various auxiliary materials in comparison**

| Auxiliary material | D [PKEK] in µm$^2$/min geometric mean | D [PKEK] in µm$^2$/min upper limit | D [PKEK] in µm$^2$/min lower limit |
|---|---|---|---|
| **1,2-propanediol** | 3.21 | 0.82 | 0.66 |
| **1,3-butanediol** | 2.99 | 1.22 | 0.86 |
| **1,2-pentanediol** | 3.48 | 1.64 | 1.12 |
| **1,2-hexanediol** | 3.66 | 0.63 | 0.55 |
| **1,2-octanediol** | 4.33 | 0.36 | 0.66 |
| **dimethyl sulfoxide** | 8.04 | 1.54 | 1.29 |
| **without auxiliary materials** | 5.04 | 1.80 | 1.32 |

**Table 3: In comparison, the geometric mean values of the permeation coefficient of PKEK in the presence of the various auxiliary materials.**

| Auxiliary material | P [PKEK] in µm/s geometric mean | P [PKEK] in µm/s upper limit | P [PKEK] in µm/s lower limit |
|---|---|---|---|
| **1,2-propanediol** | 1.27 | 0.06 | 0.05 |
| **1,3-butanediol** | 1.27 | 0.02 | 0.02 |
| **1,2-pentanediol** | 1.33 | 0.22 | 0.18 |
| **1,2-hexanediol** | 1.19 | 0.04 | 0.04 |
| **1,2-octanediol** | 1.26 | 0.08 | 0.07 |
| **dimethyl sulfoxide** | 1.29 | 0.06 | 0.06 |
| **without auxiliary materials** | 1.26 | 0.05 | 0.05 |

was not surprising, as the permeation coefficient describes how "permeable" the membrane is, i.e. which transport resistance the substance has to overcome. Since in all experiments snake skin was used as a barrier or membrane, the permeation coefficient for PKEK had to be approximately the same, unless auxiliary materials changed the permeability of the membrane. This did not seem to be the case here.

A similar picture emerged when considering the Flux or mass flow. Also here, as shown in Table 4, there were no significant differences.

**Table 4: In comparison, the geometric mean values of the Flux of PKEK in the presence of the various auxiliary materials.**

| Auxiliary material | Flux [PKEK] in [mg/(cm$^2$min)] geometric mean | Flux [PKEK] in [mg/(cm$^2$min)] upper limit | Flux [PKEK] in [mg/(cm$^2$min)] lower limit |
|---|---|---|---|
| **1,2-propanediol** | 1.16 | 0.02 | 0.03 |
| **1,3-butanediol** | 1.15 | 0.01 | 0.01 |
| **1,2-pentanediol** | 1.19 | 0.11 | 0.10 |
| **1,2-hexanediol** | 1.11 | 0.02 | 0.02 |
| **1,2-octanediol** | 1.15 | 0.04 | 0.04 |
| **dimethyl sulfoxide** | 1.17 | 0.03 | 0.04 |
| **without auxiliary materials** | 1.15 | 0.02 | 0.03 |

From the experiments, the parameters of the auxiliary materials themselves could also be determined. These are summarized in Table 5.

**Table 5: Comparison of the permeation parameters [n = 3] of a 10% auxiliary-solution in $D_2O$ through shed snake skin in the presence of PKEK at a concentration of 10%. In each case given, the arithmetic mean ± standard deviation.**

| Auxiliary material | D in x$10^{-9}$ cm$^2$/min | $t_{lag}$ in min | J in µg/cm$^2$ min$^{-1}$ | P in x$10^{-4}$ cm/min |
|---|---|---|---|---|
| **1,2-propanediol** | 1.36 ± 0.50 | 768 ± 243 | 11.93 ± 3.16 | 1.19 ± 0.32 |
| **1,3-butanediol** | 1.27 ± 0.23 | 774 ± 127 | 12.74 ± 1.85 | 1.27 ± 0.19 |
| **1,2-pentanediol** | 9.84 ± 8.13 | 602 ± 925 | 13.63 ± 3.40 | 1.36 ± 0.34 |
| **1,2-hexanediol** | 5.26 ± 2.71 | 244 ± 178 | 15.46 ± 1.94 | 1.55 ± 1.94 |
| **1,2-octanediol** | 7.09 ± 5.91 | 336 ± 403 | 0.0007 ± 0.0002 | 7.17x$10^{-5}$ ± 3.99x$10^{-4}$ |
| **dimethyl sulfoxide** | 1.24 ± 0.18 | 790 ± 121 | 26.28 ± 3.03 | 2.63 ± 0.30 |

It can be seen that the permeation coefficients of the polyhydric alkanols are similar, except that of 1,2-octanediol. That of DMSO was the largest, meaning the permeation resistance for DMSO was lower than that of the polyhydric alkanols. It also showed in the mass flow in a similar manner.

The diffusion coefficients, on the other hand, were different. Thus, the diffusion coefficient of the polyhydric alkanols increased with increasing chain length. The only exception was the 1,2-pentanediol, which had the largest diffusion coefficient. On the contrary, DMSO had a small diffusion coefficient. For all auxiliary materials, it was noted that they just not remain on the shed snake skin but overcome the skin barrier. Insofar, this must be taken into account in future formulations. The question here is, to what extent the auxiliary materials can accumulate in deeper skin layers and if this is physiologically important. According to the current safety data sheets (Safety Data Sheet 1,3 Butandiol 2017; Safety Data Sheet Dimethylsulfoxid 2017; Safety Data Sheet Propylenglycol 2015), DMSO, 1,2-propanediol and 1,3-butanediol are not classified as hazardous. Thus, these substances should be safe. For the use of enhancers in formulations, the "safety of use" is important. Lee et al.(2011) investigated to what extent the 1,2-alkanols 1,2-butanediol, 1,2-pentanediol, 1,2-hexanediol, 1,2-octanediol and 1,2-decanediol lead to skin irritations. They found that 1,2-hexanediol had the lowest objective skin irritation potential and that this ascended with increasing and decreasing chain length. However, there was no correlation between the skin absorption potential and the skin irritation potential (Lee et al. 2011). The Cosmetic Ingredient Review (CIR) Expert Panel has classified the 1,2-glycols as safe because of negative oral toxicity data and genotoxicity data. Especially pentylene glycol is used in cosmetics (Johnson et al. 2012). The Food and Drug Administration (FDA) announced for example propylene glycol to be 'generally recognized as safe' (GRAS) (GRAS Substances (SCOGS) Database 2018).

As stated at the beginning, although DMSO is a good enhancer, it requires concentrations of min. 60 %. At this high concentration, it could be that i.a. Erythema is formed and other skin irritations may occur (Anigbogu et al. 1995; Coldmann et al. 197; Kligman 1965; Notman et al. 2008). However, recent research has shown that the effect of DMSO was greatest at a concentration of 60 %, but that initial effects were already seen at concentrations of 10 %. At liposomes, meaning at multilayers of SC lipids, the influence of DMSO on the structure was examined: The penetration-promoting property of DMSO was concentration-dependent. Even with a percentage of 10 % DMSO, a decrease in the hydrogen bridge bonds at the carbonyl group of the amide and stearic acid could be determined. The order of the lipid chains of the SC lipids itself was not affected. It has been assumed that DMSO is already stored within the head groups of lipids and slightly influences their integrity. A similar picture was seen at a concentration of 30 % DMSO. Here, DMSO also accumulated within the lipids. DMSO can thus promote the penetration of hydrophilic and lipophilic substances. At 50 % DMSO, the lipid structure was already severely, but not completely damaged (Müller 2017). This shows the high stability of the lipids in the SC (Altenbach et al. 2006). In this study, the low concentration of 10 % DMSO was used since in other experiments penetration-promoting properties have already been shown at this concentration level. In a study, cutaneous application of celecoxib together with 5 % or 10 % DMSO was examined. No cytotoxic or genotoxic effects were seen *in vitro*. *In vivo*, mild irritation effects were indicated (Senna et al. 2017). Thus, a compromise was made from the minimum necessary concentration for a penetration-promoting effect and the maximum concentration required in favor of compatibility.

## 3. Discussion

All experiments were conducted on the shed snake skin of an individual and thus provide a very good comparability. It can be stated that all auxiliary materials have overcome the SC and could be determined in the acceptor. The concentration-time profile of PKEK provided the course of a saturation curve regardless of the added auxiliary material. The auxiliary materials, however, showed different courses of curves. The permeation coefficient and Flux of PKEK were not affected through the auxiliary materials. Only the diffusion coefficient of PKEK changed in the presence of DMSO - it became larger, so that PKEK permeated faster in and through the shed snake skin. For all other auxiliary materials, the PKEK diffusion coefficient was not significantly different from the PKEK diffusion coefficient without the addition of an auxiliary material. Further experiments with a larger number of samples would be necessary to confirm this.

Also, in this experiment it has been shown that even 10 % DMSO is sufficient to significantly influence the penetration of a hydrophilic active substance. Although it does not affect the extent, but it affects the speed at which the active substance enters the skin.

Since the 1,2-alkanediols are currently classified as safe and in DMSO the skin irritation potential in minimal concentrations is low, these auxiliary materials are quite interesting.

## 4. Experimental

### 4.1. Materials

l-Prolyl- l -lysyl- l -α-glutamyl- l -lysin (PKEK) was generously donated by Evonik Industries AG (Essen, Germany). Deuterium oxide ($D_2O$) was purchased by ARMAR Chemicals GmbH (Leipzig, Germany). 1,2-Propanediol, 1,3-butanediol and dimethyl sulfoxide (DMSO) was supplied by Fagron GmbH & Co. KG (Barsbüttel, Germany). 1,2-Pentanediol, 1,2-hexanediol and 1,2-octanediol were used from Symrise AG

(Holzminden, Germany). Polyglycerol-4-isostearate (Hydriol® PGI) was acquired from Hydrior AG (Wettingen, Switzerland). Distilled water was taken from own production in the Institute of Pharmacy at Martin Luther University Halle-Wittenberg (Halle (Saale), Germany).

The shed snake skin of *Pantherophis guttatus* was donated by Zoologischer Garten Halle GmbH (Halle (Saale), Germany).

### 4.2. *Preparation of snake skin*

The skin of *Pantherophis guttatus* has served as a model membrane. The shed snake skin was removed from the terrarium after the snake had molted. It was stored at room temperature. Before the experiment started, the shed snake skin was cut into slices. Especially the skin at the abdomen was used because the "honeycombs" of stated area are larger and thus fit better to the dimensions of the Teflon cell.

### 4.3. *Measurement of skin thickness*

For the calculation of the pharmacokinetic parameters, the thickness of the skin was needed. Prior to the experiment, the skin thickness was measured in triplicate and arithmetically averaged. For this, the Coating thickness gauge MT25B from Dr. Johannes Heidenhain GmbH (Traunreut, Germany) was used.

### 4.4. *Recording the IR spectra of the potential auxiliary and model peptides*

Using the FT-IR spectrometer IFS 28 from Bruker Optik GmbH (Ettlingen, Germany) and an ATR measuring unit from Thermo Spectra-Tech (Shelton, USA), the IR spectra were recorded.

Conducting the experiment, a Fresnel ATR crystal of ZnSe (angle of incidence 45°) from Thermo Electron Corporation (Waltham, USA) was used.

With the following constant parameters the spectra were recorded:

| | |
|---|---|
| IR-Source | Globar (MIR) |
| Detector | DTGS |
| Sensitivity | 2cm $^{-1}$ |
| Number of sample scans | 32 |
| Number of background scans | 32 |
| Phase resolution | 128 |

The data was recorded with the OPUS software (Bruker Optik GmbH, Ettlingen, Germany), version 4.2.

For PKEK and each auxiliary material, a calibration line in the range of 0,2 % to 10 % was determined.

### 4.5. *Permeation experiments*

The permeation experiments were conducted the same way as described in the preceding paper "Dermal Peptide Delivery Using Enhancer Molecules and Colloidal Carrier Systems – Part IV[#]: Search for an alternative model membrane for future ATR permeation studies using PKEK as model substance": They were performed at the IFS 28 and an ATR measuring unit designed by Thermo Spectra-Tech (Shelton, USA) with the Teflon diffusion cell developed by Hartmann et al. (2004) and modified according to Guenther (2010). The Teflon diffusion cell has a membrane between a donor and acceptor compartment. In order that the IR beam can penetrate 1-2 µm into the acceptor medium, the acceptor had direct contact with the zinc selenide crystal. An "online" measurement is possible, meaning, in real time the increase in concentration in the acceptor can be tracked. Unfortunately, at an acceptor volume of 50 µl, a realization of *sink* conditions was not possible. The formulation was added in excess to the donor, to achieve a saturation equilibrium in the acceptor. In foregoing experiments, the acceptor dried out (Guenther 2010). In prevention of this, the lid of the Teflon diffusion cell was closed with cotton wool which was soaked in $D_2O$. Despite this procedure, the test duration was limited to 36 h.

The shed snake skin was hydrated in $D_2O$ for 3 h before the experiment started. At first, the ATR unit was tempered to 32 °C and the sample room, as well as the spectrometer was rinsed with dry, low carbon dioxide air. The respective formulation was freshly prepared for the permeation experiment. $D_2O$ was used to produce a 10 % PKEK with 10 % auxiliary material. Primarily, the PKEK was weighed (scale METTLER TOLEDO XA 105 Dual Range (Mettler Toledo, Gießen, Germany)) and with $D_2O$ a 10 % solution was prepared. If an auxiliary material was a component of the formulation, a 10% solution was produced with the auxiliary, which was used to dissolve the weighed out PKEK.

Foremost, the acceptor plate was mounted on the ZnSe ATR crystal and a background measurement was taken. This was followed by adding 50 µl $D_2O$ to the acceptor plate of the Teflon measuring cell, or in other words, pipetted on the acceptor and this was gauged as the first sample. In the next step, the snake skin was placed carefully and bubble-free on the $D_2O$ and mounted on top the upper part of the Teflon measuring cell. The ZnSe ATR crystal together with the Teflon measuring cell was placed on the ATR unit and 50 µl of the formulation was pipetted onto the skin and the Teflon measuring cell was sealed (including the filter paper soaked with $D_2O$, which serves as protection against evaporation). After the first sample measurement, zero defined as point of time, the multiple measurement was started: 36 measurements every 300 s, 36 measurements every 600 s, 36 measurements every 900 s, 36 measurements every 1800 s and 36 measurements every 3600 s. After the experiment, the Teflon measuring cell was disassembled and cleaned with water and ethanol and dried with compressed air.

**Table 6: Composition of the tested formulations**

| Formulations | Composition |
|---|---|
| **Formulation 1** | 10 % PKEK in $D_2O$ |
| **Formulation 2** | 10 % PKEK and 10 % 1,2-Propanediol in $D_2O$ |
| **Formulation 3** | 10 % PKEK and 10 % 1,3-Butanediol in $D_2O$ |
| **Formulation 4** | 10 % PKEK and 10 % 1,2-Pentanediol in $D_2O$ |
| **Formulation 5** | 10 % PKEK and 10 % 1,2- Hexanediol in $D_2O$ |
| **Formulation 6** | 10 % PKEK and 10 % 1,2-Octanediol in $D_2O$ |
| **Formulation 7** | 10 % PKEK and 10 % Dimethyl sulfoxide in $D_2O$ |

The evaluation of spectra was done with the software OPUS 4.2 spectroscopy software from Bruker Optik GmbH (Ettlingen, Germany). First of all, an atmospheric compensation was performed. Afterwards, the peak areas were integrated and data was evaluated mathematically.

As described in the paper "Dermal Peptide Delivery Using Enhancer Molecules and Colloidal Carrier Systems – Part IV[#]: Search for an alternative model membrane for future ATR permeation studies using PKEK as model substance", each substance provides a specific ATR spectrum, at a mixture of substances a spectrum represents the interactions of the various substances with one another. Again, a specific peak was necessary for the respective substance.

An overlap (even partial) of these peaks had to be excluded through the uptake of comparative spectra of pure substances. This revealed, which peak of the mixture belonged to which substance. In which areas the auxiliary material (that is to be examined) and the used solvent $D_2O$ are not to oscillate, are stipulated by PKEK which acts as a model of a medicinal agent. As reverse conclusion, it was not possible to investigate the effect of any auxiliary material with PKEK as a model of a medicinal agent. Fig. 4 illustrates this. On the other hand, PKEK showed an evaluable peak in the 1700-1730 cm$^{-1}$ range which was not overlapped by either 1,2-pentanediol or $D_2O$. Also in this experiment, as described earlier, $D_2O$ was used as a solvent, since here significant oscillations of PKEK at 1630 cm$^{-1}$ would have also been overlapped by $H_2O$. An unequivocal identification and quantification of PKEK and the respective auxiliary materials were possible through time-dependent increase in identity of each substance specific band.

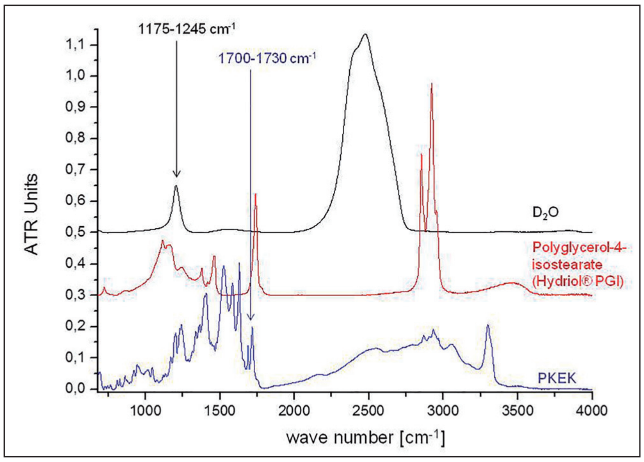

Fig. 4: In comparison ATR spectra of $D_2O$, Hydriol®PGI and PKEK are shown. The Hydriol®PGI shows a peak in the region of the spectrum which is to be used for the evaluation of PKEK. Consequently, the penetration of PKEK in a sample containing Hydriol®PGI can be not examined. For clarity, the spectra have been moved on the y-axis.

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Conflicts of interest: None declared

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