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Summary of the Ph.D. thesis

Investigation of new innovative techniques for modeling skin permeation

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1. INTRODUCTION

Dermal and transdermal formulations are commonly used for carrying drugs to the skin and the underlying tissue, or through the skin for systemic action. In recent years, the number of dermal and transdermal formulations has increased. According to a business study carried out in 2016, the profits from transdermal preparations will rise dramatically by 2024. The main reason for their popularity is that they have a lot of advantages. For example, avoiding the first pass metabolism of the liver, gastrointestinal tract protection and non-invasive application. The growing market demands require an integrated regulatory environment that can easily identify and evaluate the product properties anywhere in the world and facilitate the research of drug optimization of the penetration through human skin.

Modeling of permeation through the skin is a complex challenge. There are various *in vitro* and *in vivo* methods. The different methods, along with the properties of the product, influence how the system can be tested most effectively. The investigational method is greatly influenced by the predictive ability, time, and labor requirements of the given method and its cost.

In vitro permeation studies using well-defined diffusion cells, skin models and membranes can be useful tools in the design and optimization of skin formulations. The most commonly used quantitative method for measuring *in vitro* skin permeation is the use of Franz diffusion cell described by numerous directives. In this method the test formulation is placed on the surface of a skin model, which is positioned as a barrier between the donor compartment and the receptor compartment of the Franz diffusion cell. Advantages of the Franz cell method are that measurements can be carried out on human skin samples among other potential membranes; multiple tests can be performed; several formulations can be tested at the same time; there is no need for radio-labelling of the test material; and there are no ethical issues.

Human skin examinations give the most appropriate information, but, because of their high cost, it is a commonly accepted way to choose simpler *in vitro* methods in the early stages of formulation development. In addition to pharmaceutical research, these investigations also help other industries. In agrochemistry, pesticides and insect repellents are involved, and the veterinary and cosmetic industries also rely on these methods.

2. EXPERIMENTAL AIMS

The aim of this research was to investigate the applicability and substitutability of traditionally used and new, innovative and promising methods for modeling skin permeation. During the work we aimed to:

- compare the Hanson Franz cell and the Logan Franz cell and their substitutability;
- examine the applicability of different membranes, in particular whether the human epidermis can be replaced by a synthetic membrane;
- examine the applicability of Skin PAMPA method;
- investigate Raman mapping as a semiquantitative method; and
- examine the sensitivity of the methods to detect differences between different formulations.

Dermal preparations such as a hydrogel, different types of creams and a nanostructured lipid carrier gel were investigated to compare different methods. The following steps were set.

In the first part of my Ph.D. work (Experimental part 1), different quantitative skin permeation modeling methods were used to study drug release and permeation. Two types of vertical Franz diffusion cells (Logan, Hanson) were compared by three different membranes, including cellulose, Strat-M and the gold-standard heat-separated human epidermis. Next, these cells were compared to the Skin PAMPA method, as well.

During the second part of my work (Experimental part 2), I aimed to investigate whether there is correlation between the findings of quantitative experiments and semiquantitative spectroscopic measurements. Logan Franz cell, skin PAMPA and Raman spectroscopic methods were compared. Another aim was also to determine how selective these methods are in order to be able to demonstrate the differences between different compositions. A hydrogel and two types of creams were investigated as these are the most generally used dermal preparations.

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Lidocaine Containing Nanostructured Lipid Carrier Gel (LID-NLC gel)

Lidocaine base, glycerol, Cremophor® RH 60, Apifil®, Methocel™ E4M and purified water were used.

3.1.2. Diclofenac Sodium (DFNa) Containing Formulations

Diclofenac sodium, ethanol 96 *w/w%*, cetostearyl alcohol, liquid paraffin, white petrolatum, white beeswax, wool fat, oleyl oleate and castor oil, polysorbate 60, MethocelTM E4M and purified water were used.

3.1.3. Synthetic and Biological Membranes

The cellulose acetate membrane (Porafil membrane filter, cellulose acetate, pore diameter: 0.45 μm), Strat-M membrane (Strat-M Membrane, Transdermal Diffusion Test Model, 25 mm) and skin PAMPA sandwiches (with UV plates) were used as synthetic membrane. Excised human skin was obtained from Caucasian female patients by a routine plastic surgery.

3.2. Methods

3.2.1. Preparation of the Lidocaine Containing Nanostructured Lipid Carrier Gel (LID-NLC gel)

NLCs are colloidal carriers which were introduced in the early 1990s. They are derived from o/w emulsions by replacing the liquid lipid with a solid lipid at room temperature. The lipophlic phase of NLC included Apifil, Cremophor RH 60 and Miglyol 812 N, which were melted at 60 °C under controlled stirring. Then lidocaine was added to the melted lipid phase under similar conditions. Then warm purified water was added to the lipid phase to form the pre-emulsion. The pre-emulsion was ultrasonicated using a Hielscher UP200S compact ultrasonic homogenizer for 10 min at 70 *w/w%* amplitude. At the end, the sample was cooled in ice to obtain the solid lipid particles (LID-NLC). For dermal application, a concentrated gel was formed at room temperature with glycerol and Methocel E4M. In the last step, the NLC dispersion was added to the gel (LID-NLC gel) to form the final composition (5 *w/w%* of lidocaine). A blank-NLC and blank-NLC gel were also prepared using the same procedure, but without adding lidocaine. Table 1 summarizes the formulations.

Table 1 *Compositions of the test preparations*

LID-NLC	LID-NLC Gel
Apifil	LID-NLC
Cremophor RH60	Glycerol
Miglyol 812N	Methocel E4M
Purified water	
Lidocaine	

3.2.2. Preparation of the Diclofenac Sodium Containing Formulations

Three different semisolid preparations were formulated (Table 2). The conventional hydrogel was prepared with 1 *w/w%* DFNa dissolved in the mixture of purified water and ethanol 96 *w/w%*, then Methocel E4M and microbiological preservative were added. In the

case of the o/w cream, the oily phase consisting of cetostearyl alcohol, liquid paraffin, white petrolatum and Polysorbate 60 was heated up to 60 °C. Then, hot water was added to the oily phase under agitation. DFNa was dispersed in the preparation and it was homogenized until the mixture was cooled. Finally, the microbiological preservative was added. In the case of the w/o cream, the oily phase consisting of white beeswax, wool fat, oleyl oleate and castor oil was heated up to 60 °C. Then, hot water was added to the oily phase under agitation. Finally, DFNa was dispersed in the preparation and it was homogenized.

Table 2 *Compositions of the diclofenac sodium containing formulations.*

hydrogel	o/w cream	w/o cream
Diclofenac sodium	Diclofenac sodium	Diclofenac sodium
Methocel E4M	Cetostearyl alcohol	White beeswax
Ethanol 96 *w/w%*	Liquid paraffin	Wool fat
Purified water	Purified water	Purified water
	White petrolatum	Castor oil
	Polysorbate 60	Oleyl oleate

3.2.3. Drug Release and Permeation Studies

Three different methods were used to model and compare drug release and diffusion through the membrane and permeation through the skin from different formulations (Table 3).

Table 3 *The experimental design of drug diffusion and permeation studies.*

	Hanson Microette TM Topical&Transdermal Franz Diffusion Cell System	**LOGAN Automated Dry Heat Sampling System**	**Skin PAMPA**
Static or dinamic cell	static	static	static
Number of cells	6	6	96
Cell properties	closed (under pressure)	open	open
Permeation surface area	1.76 cm^2	1.76 cm^2	0.3 cm^2
Amount of acceptor phase	7 ml	9 ml	250 µl
Acceptor phase	Phosphate buffer solution pH 7.4	Phosphate buffer solution pH 7.4	Phosphate buffer solution pH 7.4
Donor phase	0.30 g	0.30 g	70 µl
Membrane	Synthetic • cellulose • Strat-M Biological • HSE	Synthetic • cellulose • Strat-M Biological • HSE	Skin PAMPA membrane
Temperature	32 °C	32°C	32°C
Sampling	automatic	automatic	by hand
Time of measurement	6 or 24 h	6 or 24 h	6 h
Determination of active substance	Spectrophotometry	Spectrophotometry	Spectrophotometry

The methods included two types of vertical Franz diffusion cells, namely Hanson Microette TM Topical & Transdermal Diffusion Cell System, and Logan Automated Dry heat sampling system. The third method used was the skin PAMPA method. In the Franz cells, the donor and acceptor phases were separated by either a synthetic membrane cellulose acetate and Strat-M membrane or a biological membrane: heat-separated human epidermis (HSE). The heat-separation method was applied to isolate the epidermis. The excised human fat-free subcutaneous skin was put in a water bath (60±0.5 °C, 1 minute), and separated the epidermis from the dermis. The skin PAMPA sandwiches were used after a 24-hour hydration. Permeation profiles of dermal formulations were obtained. The cumulative amount (Q) of drug permeated per cm^2 at final time point was calculated. The flux (J) was the slope of the cumulative amounts of API ($\mu g/cm^2$) permeated versus time (h) profiles. Time point correlations between the amounts of drug permeated through heat-separated human epidermis and skin PAMPA membrane were shown and correlation coefficients (R^2) were calculated.

3.2.4. Investigation of Skin Permeation with Raman Spectroscopy

Excised human subcutaneous fat-free skin (epidermis and dermis) was used. It was obtained from Caucasian female patients who underwent abdominal plastic surgery. 1 cm^2 of the skin surface was treated with the formulations for 3 h at 32 °C. The treated skins were frozen and sectioned (10-μm-thick cross-sections) with a Leica CM1950 cryostat. The microtomed skin samples were placed on an aluminum surface with the SC towards the top of the plate. Raman spectroscopic measurements were made with a Thermo Fisher DXR Dispersive Raman Spectrometer equipped with a CCD camera and a diode laser. A laser light source of 780 nm wavelength was used, with a maximum power of 24 mW, which is the best source for studying biological samples. With the use of this type of laser source, fluorescence had less effect. The microscopic lens used for the measurements was magnified by 50 × and the pinhole aperture was 25 μm. A 200 to 1.800 μm area was explored in the case of chemical mapping; the step size was 50 μm vertically and horizontally. 205 spectra were observed, 16 scans were reported to accumulate each spectrum, and the exposure period was 2 s. When analyzing the treated vs. untreated skin samples, the different spectra of each component of the formulations and drug were used as reference points. A laser light of 532 nm was used to record the different spectra of the components and formulations. 32 scans were registered for each spectrum, with an exposure time of 6 s. The optics magnitude in the Raman microscope was 10 with a 25 μm slit aperture. Data acquisition and analysis were accomplished using OMNICTM8.2 for Dispersive Raman software package.

3.2.5. Statistical Analysis

Data analysis, statistics and graphs were performed from the experimental data via Microsoft® Excel® (Microsoft Office Professional Plus 2013, Microsoft Excel 15.0.5023.100, Microsoft Corporation, Washington, USA), OriginPro® 8.6 software (OriginLab® Corporation, Northampton, Massachusetts, USA). Prism for Windows (GraphPad Software Inc., La Jolla, CA, USA) was used to conduct statistical data analysis using two-way ANOVA variance analysis (Bonferroni post-test). Differences were regarded as significant if *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ and ****$p < 0.0001$ versus the control.

4. RESULTS AND DISCUSSION

4.1. Experimental part 1

Experimental part 1 contains the comparison of two types of Franz cells using different membranes and comparison of Franz cells method to skin PAMPA method. The 5 *w/w%* lidocaine containing NLC gel was used as test preparation. The amount of lidocaine released/permeated *in vitro* from the LID-NLC gel through the two synthetic and one biological membrane was calculated in terms of mean cumulative amount in $\mu g/cm^2 \pm$ SD by 24 h.

Cellulose acetate membrane was used for *in vitro* release tests (IVRT) and heat-separated human epidermis was used for *in vitro* permeation tests (IVPT). The findings of both approaches were compared with the Strat-M membrane, which is a synthetic membrane specifically designed for skin penetration tests.

The findings are shown in Figure 1, which demonstrates the results obtained after 24 hours. There was a significant difference between the results of the two cells in the case of the cellulose membrane and significant difference was observed in the case of Strat-M and HSE membranes, too. The drug release through the synthetic cellulose membrane is significantly higher than permeation through the biological HSE membrane. It is important to know the extent of release (IVRT), but this does not provide relevant information for permeation. It is very important to examine the permeation process through the skin (IVPT) to know not only the released amount of drug, but also to see the interactions between the drug or the drug delivery system and the skin. However, it is promising that the results of synthetic Strat-M membrane are very close to the result of HSE.

For skin PAMPA measurements, we found that the integrity of the membrane was insufficient for 24-hour measurements, that is why skin PAMPA measurements were only run for a maximum of 6 hours.

The *in vitro* released/permeated amount of lidocaine from the LID-NLC gel through the two synthetic and one biological membrane and skin PAMPA were calculated in terms of mean cumulative amount in µg/cm^2 ± SD by 6 h.

The results were compared with the 6 h data obtained on Franz cells (Figure 2). There was no significant difference between the results of the skin PAMPA membrane, Logan Strat-M and Logan HSE membrane.

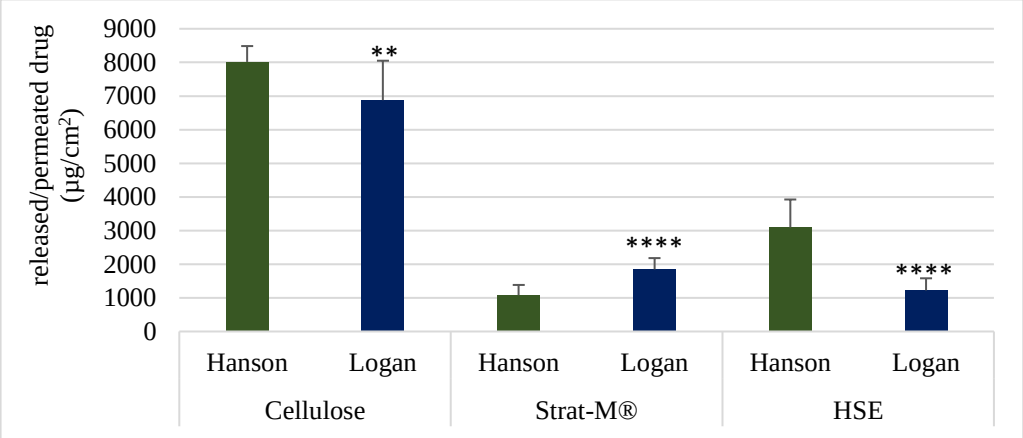

Figure 1 *Comparison of diffusion cells and membranes (**** p <0.0001, **p < 0.01)*

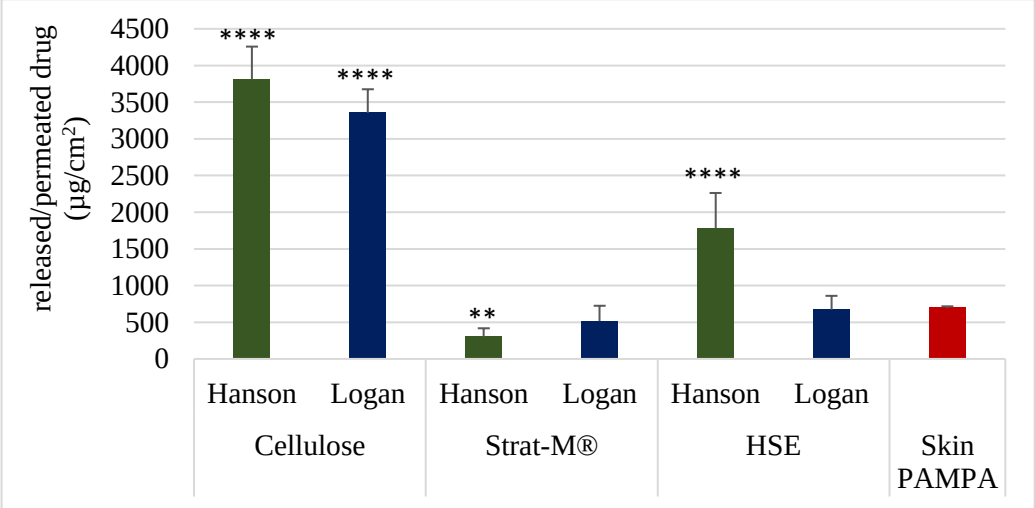

Figure 2. *Comparison of skin PAMPA membrane results with Franz cell measurements (**p < 0.01, **** p <0.0001 vs skin PAMPA)*

The following figures illustrate the results evaluated separately on the Hanson and Logan cells (Figure 3-4). The analysis of relevance for the HSE membrane, the Strat-M membrane and the skin PAMPA membrane did not show a significant difference in the Logan cell. Significantly, more drugs pass through the cellulose membrane over 6 hours. The results obtained from the Hanson instrument showed a higher standard deviation. Compared to the HSE membrane, the findings of all three other membranes showed a significant difference at the end of the 6-hour study.

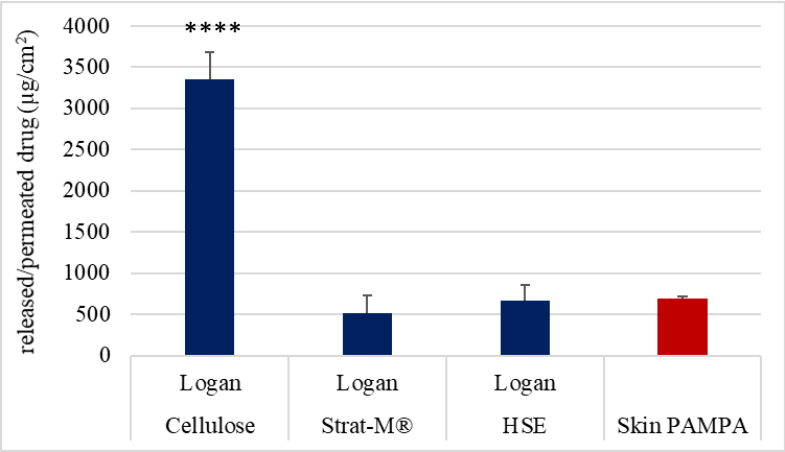

Figure 3 *Results of Logan cell and skin PAMPA (**** p <0.0001 vs Logan HSE)*

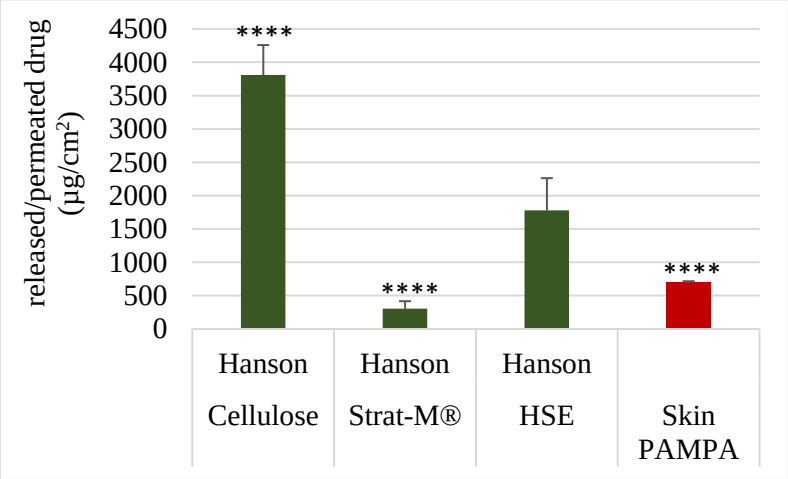

Figure 4 *Results of Hanson cell and skin PAMPA (**** p <0.0001 vs Hanson HSE)*

Summary of experimental part 1

A lidocaine-loaded, NLC gel were used to study *in vitro* release and skin permeation. Two types of Franz cell equipment were compared to each other. Logan and Hanson Franz cells provided different results.

Different membranes were compared, as well. Both the new special skin PAMPA membrane and new synthetic Strat-M membranes correlated well with the HSE membrane and the Strat-M membranes showed the most similar drug permeability profile to the *in vitro* human skin membrane.

Based on the results, it can be stated that the IVPT tests approved in the guidelines (penetration of the human epidermis) can be better replaced with a Strat-M membrane patterned on a Logan cell and a skin PAMPA device. The results of the Hanson cell showed

a significant difference between the different membranes and the skin PAMPA. In our work, further investigations were performed on the Logan cell.

4.2. Experimental part 2

In experimental part 2, Logan Franz cell and skin PAMPA methods were compared to Raman spectroscopy. Logan Franz cell and skin PAMPA are quantitative, while Raman spectroscopy is semiquantitative. The sensitivity of these methods was also tested by the investigation of different semisolid preparations.

Three most commonly used dermal formulations were compared: a hydrogel, an o/w and a w/o cream each of them containing 1 *w/w%* of DFNa.

4.2.1. Investigation of Different Semisolid Formulations by Quantitative Methods

The Franz cell and skin PAMPA methods are based on the quantitative measurement of drug permeation through a skin-mimicking membrane. Figures 5 and 6 show the cumulative amount of drug passing through the different membranes from the different types of formulations in 6 h in $\mu g/cm^2$.

In Figure 5 different formulations were compared. In the case of cellulose membrane, the hydrogel showed the highest drug release values. The hydrogel is an aqueous-based system where the drug is in dissolved form, and diffusion through the synthetic membrane is high. The highest permeation from the o/w cream was observed in HSE, Strat-M and Skin PAMPA measurements because DFNa is in the outer aqueous phase of the cream, and the emulsifier content of the cream promotes permeation through human skin and special skin-mimicking membranes. The release and permeation of DFNa from the w/o cream were extremely low in all measurements. The drug is presumably in the inner phase of the cream, and the low diffusion and permeation can be explained by the fact that the diffusion of DFNa through the oil phase limits the release of the drug.

In the case of the hydrogel and o/w cream, the other membranes showed a significant difference compared to the result of HSE, but the result of the Strat-M membrane was closest to that of the human epidermis. In the case of the w/o cream, there was no significant difference between the results of HSE and Strat-M membrane.

In Figure 6, we examined the different compositions on the different membranes. Through the cellulose membrane, the hydrogel showed the best results, followed by the o/w cream and finally the w/o cream. There was a significant difference in drug release between the formulations. Penetration through the human epidermis changed the order, the best result was given by the o/w cream, followed by the hydrogel and then the w/o cream. A possible explanation is that the emulsifiers in the o/w cream have a penetration enhancing effect,

which facilitated the penetration of the active ingredient. On the Strat-M membrane and skin PAMPA membrane, the sequence followed the results obtained on the HSE. It can be concluded that the PAMPA membrane, containing the compounds of the stratum corneum, and the Strat-M skin-mimic membrane correlates well with the HSE.

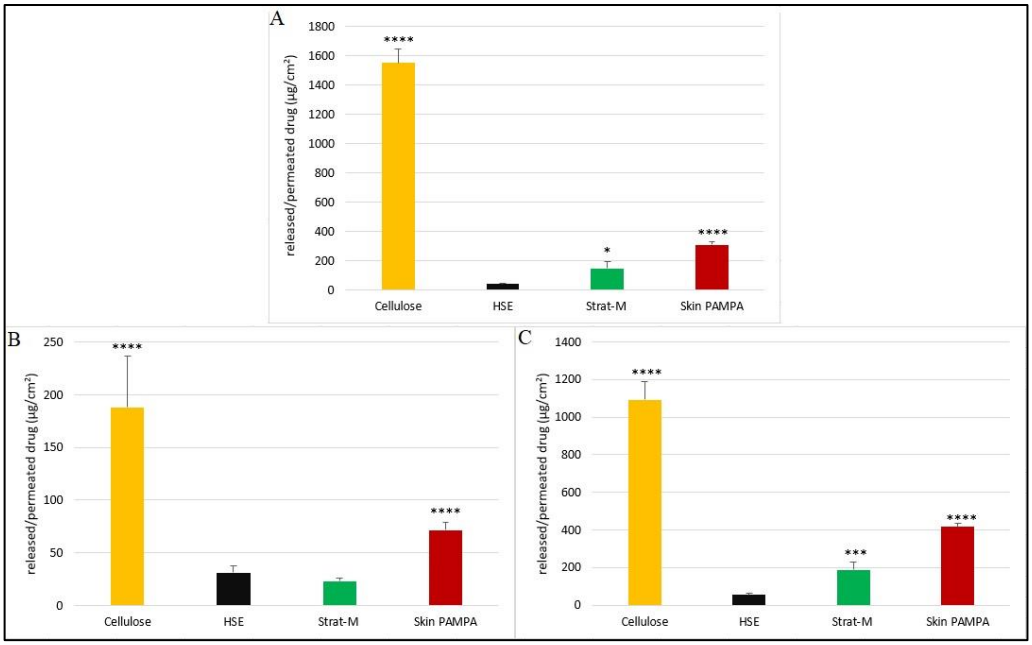

Figure 5 A**: Results of drug released and penetrated from the hydrogel **B**: Results of drug released and penetrated from w/o cream **C**: Results of drug released and penetrated from o/w cream (* p <0.0001, ***p < 0.001, * p <0.05 vs HSE)*

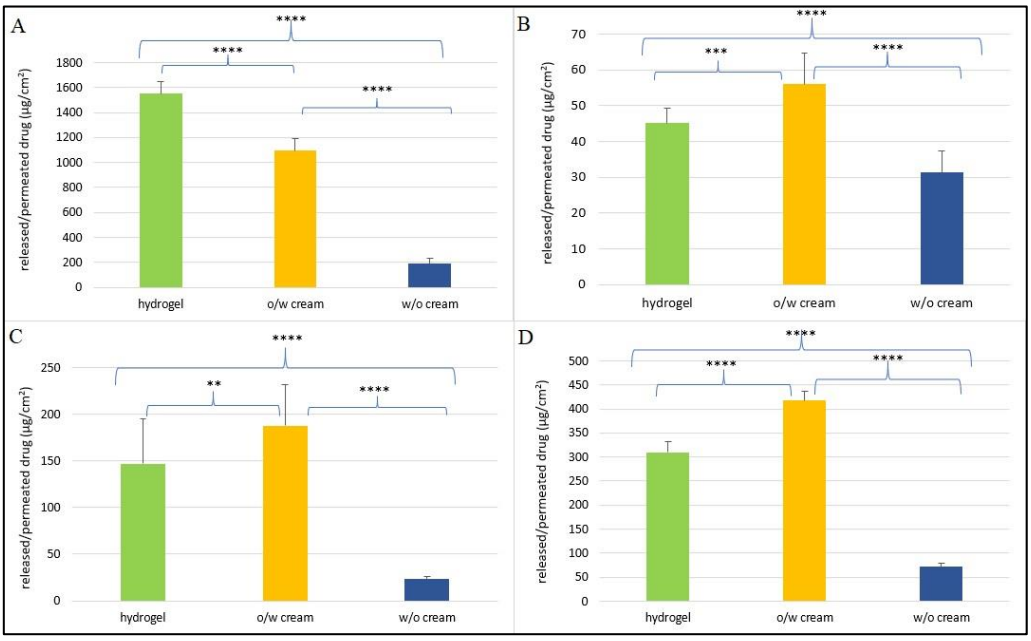

Figure 6 A** Results of drug diffusion through cellulose membrane **B** Results of drug penetration through the HSE membrane **C** Results of drug penetration through Strat-M membrane **D** Results of drug penetration through skin PAMPA membrane (* p <0.0001, ***p < 0.001, ** p < 0.01)*

The mathematical evaluation of the results is shown in Table 4. Permeation parameters (Q, and J) show the differences between the methods and formulations. All methods have good sensitivity to show significant differentiation between the different formulations, so it is a very good tool in the preformulation phase to find the best suited one for the purpose of use.

Table 4 *Permeation parameters of diclofenac sodium through different membranes after 6 h.*

Formulation	Q, 6 h (µg/cm^2)	J (µg/cm^2/h)
	cellulose	
hydrogel	1552.0 ± 98.9	76.03
o/w cream	1092.9 ± 98.4	179.13
w/o cream	188.2 ± 48.8	31.36
	Strat-M	
hydrogel	146.83 ± 47.94	23.593
o/w cream	187.68 ± 44.33	29.966
w/o cream	23.17 ± 3.04	3.3194
	HSE	
hydrogel	45.18 ± 4.15	7.32
o/w cream	56.1 ± 8.6	8.37
w/o cream	31.4 ± 6.0	4.50
	skin PAMPA	
hydrogel	310.1 ± 21.4	52.59
o/w cream	417.6 ± 19.7	68.51
w/o cream	71.5 ± 7.1	11.85

Q, cumulative amount of diclofenac sodium permeated per cm^2 at 6 h (mean ± SD, n = 6); **J,** flux determined from the slope of the cumulative amounts of diclofenac sodium permeated (µg/cm^2) versus time (h) profiles

Figures 7 and 8 show the correlation of the skin PAMPA and Strat-M membrane to HSE. Both synthetic membranes demonstrate high correlation to HSE in this study. The time point correlation between the synthetic membranes and HSE was in the range of 0.93–0.99.

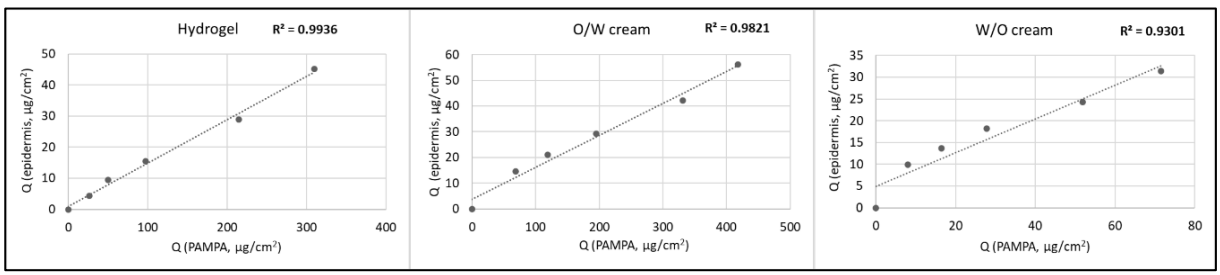

Figure 7 *Diclofenac sodium permeation, time point correlations between the amounts of drug permeated through heat-separated human epidermis and skin PAMPA*

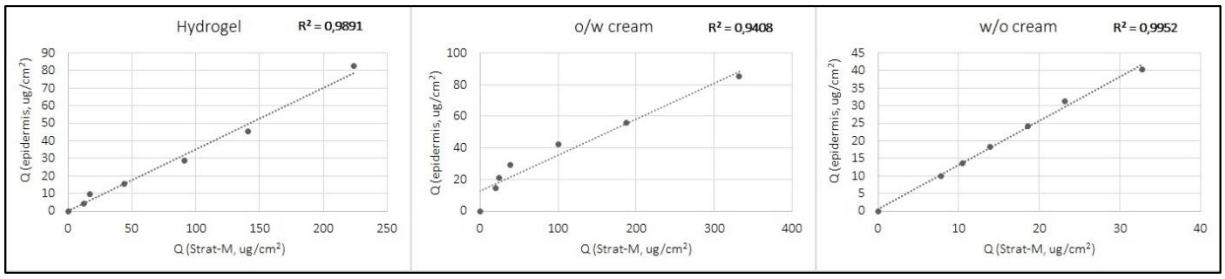

Figure 8 *Diclofenac sodium permeation, time point correlations between the amounts of drug permeated through heat-separated human epidermis and Strat-M membrane*

4.2.2. Semiquantitative study: RAMAN Mapping

The Raman correlation map proves the presence of the permeated drug formulations in the different regions of the human skin, from the skin surface to the lower layers of the dermis after treatment with the different compositions. The Raman spectra of the skin are really diverse and consist of numerous bands originating from different skin segments (e.g., nucleic acids, lipids, proteins) [87,97,98]. Several bands are overlapping with the spectra of the examined preparations. During the Raman experiments, the differences in the localization of the formulations in the skin regions were determined and compared with the Franz cell and skin PAMPA results.

The correlation maps, which showed the distribution of DFNa, were produced by fitting the appropriate spectra to the spectra of the treated skin. DFNa is easily determined from the formulations but the intensities of the characteristic DFNa peaks are very low. Therefore, the spectra of the pure API could not be used to make an acceptable correlation map. In this case, we had to use the spectrum of the whole preparation to make the skin distribution correlation maps, which indicates the presence of DFNa as well. The spectral maps were resolved in order to verify the presence of the formulation in the different regions of the human skin. The fingerprint region of the preparation spectra was related to the spectra of human skin being tested and untreated. The similarity was shown as intensity. The distribution profiles describing the relationship between the map spectra (treated skin specimen) and the defined reference spectrum (fingerprint region) were created. The resulting correlation intensity values of the map spectra are similar to the match values of the reference spectra. A more powerful intensity rate means a higher correlation with the reference spectrum.

The Raman chemical maps of the preparations are shown in Figure 9. In the case of the hydrogel, the most permeated drugs are found in the upper layers of the skin, the epidermis and the upper dermis. The o/w cream mostly permeated into the deeper layers of

the skin. This is due to the emulsifier, which increases permeation. In the case of the w/o cream, most of the composition could be found only in the stratum corneum region, and deeper permeation was blocked. This is due to its really high oil content in the external phase, which cannot pass through the hydrophilic layer of the epidermis.

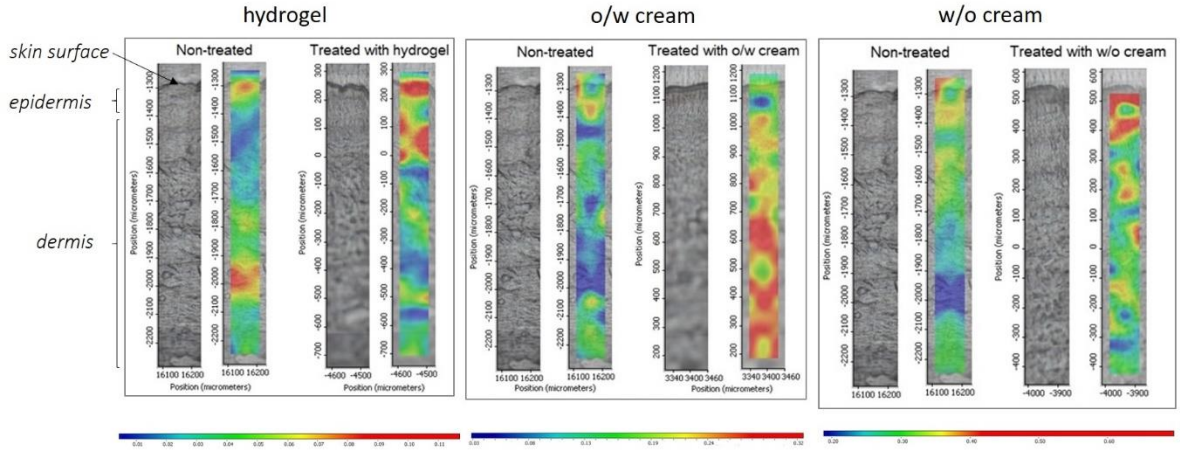

Figure 9 *Raman correlation maps for the distribution of diclofenac sodium in human skin after treatment with hydrogel, o/w cream and w/o cream. Untreated skin is also displayed as a control in all cases.*

Color coding of drug formulation content: red > yellow > green > blue.

These results correlate well with the results of HSE and skin PAMPA. In correlation with these results, the o/w cream shows the most effective permeation results where the formulation could be found in the dermis, followed by hydrogel, where the formulation passed through the regions of the epidermis and dermis. The permeation of the w/o cream was the lowest with all the methods during the time of the experiment.

Summary of experimental part 2

The investigations proved the applicability of the Strat-M membrane and the skin PAMPA method in skin permeation tests. Both methods correlated highly with the HSE membrane the permeation of different dermal formulations was compared. However, the quantity of the permeated drug from different formulations was different.

The comparison of the results indicates that the skin PAMPA and Strat-M method were closer to the gold standard HSE (IVPT) method. Its use before IVPT tests may be beneficial as it shows differences between formulations in the same way as HSE.

This part of the work also highlights the capability of Raman spectroscopy as a non-destructive technique for studying skin distribution of active ingredients and following the active ingredient in the skin layers. It could semi-quantitatively estimate the relative amounts of preparations permeated into the different skin layers. It is important to understand how

different formulations influence the permeation of active agents into/through the skin as this presents relevant information for formulation developers. In the current study, the results of Raman mapping have high correlation with the results of Strat-M, skin PAMPA and IVPT methods.

THESIS FINDINGS

I. Comparative study of diffusion cells

- There was significant difference between the results of the two types of Franz cell devices.
- The amount of drug permeated on each device was dependent on the membrane. For devices alone, no general correlation can be created.
- These findings should be considered for official assessment and authorization.

II. Investigation of Strat-M synthetic membrane

- The Strat-M membrane findings were compared to the already well-defined cellulose membrane and heat-separated human epidermis.
- Based on the results, significantly less active substance passed through the innovative synthetic Strat-M membrane compared to the cellulose membrane. The Strat-M membrane showed almost the same result as the values measured on HSE due to its special skin-mimic properties. Examining the 24-hour cumulative amounts measured on the HSE, the Strat-M membrane also showed a good correlation with the human epidermis in formulations of different compositions. The penetration-enhancing effect of the o/w cream, which is well detectable on HSE, could be well modeled by the Strat-M membrane.
- Based on these results, the synthetic Strat-M membrane can be recommended for IVPT measurements to replace the human epidermis, thus eliminating the disadvantageous properties of the biological membrane.

III. Investigation of Skin PAMPA method

- Compared to the cellulose membrane, significantly less drug was permeated on the skin PAMPA membrane, but significantly more drug was permeated compared to the tests performed on HSE. The skin PAMPA membrane proved to be more permeable in terms of cumulative amount of drug than the Strat-M membrane.

- In the case of different formulations, the skin PAMPA membrane, like the Strat-M membrane, well modeled the results obtained at HSE. It was able to differentiate between each formulation.
- It can be concluded that the skin PAMPA method may also be suitable to replace the HSE membrane, with the limitation that only a shorter 6-hour test period is recommended to ensure membrane integrity.

IV. Investigation of Raman spectroscopy

- The Raman spectroscopy with chemical mapping was studied, which allows for the examination of penetration across the whole skin. It was already mentioned in the most recent EMA guideline, so it is critical to investigate the method's applicability.
- The results confirmed that the method is suitable for detecting differences between preparations. The penetration enhancing effect was well detectable, too.
- Raman mapping may complement the results of quantitative methods of skin penetration.

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