



University of Szeged
Faculty of Pharmacy
Department of Pharmaceutical Technology

Summary of the Ph.D. thesis

Investigation of the skin barrier function and transdermal drug delivery techniques

Boglárka Balázs

Supervisors:

Dr. habil. Erzsébet Csányi
Dr. Szilvia Berkó Ph.D.

Szeged

2016

University of Szeged
Doctoral School of Pharmaceutical Sciences

Educational Program: Pharmaceutical Technology
Head: Prof. Dr. Piroska Szabó-Révész D.Sc.

Department of Pharmaceutical Technology

Supervisors:

Dr. habil. Erzsébet Csányi

Dr. Szilvia Berkó Ph.D.

Boglárka Balázs

INVESTIGATION OF THE SKIN BARRIER FUNCTION AND TRANSDERMAL DRUG DELIVERY TECHNIQUES

Final Exam Committee

Head: *Prof. Dr. István Erős D.Sc.*, University of Szeged, Department of Pharmaceutical Technology

Members: *Dr. István Zupkó Ph.D.*, University of Szeged, Department of Pharmacodynamics and Biopharmacy
Dr. István Antal Ph.D., Semmelweis University, Department of Pharmaceutics

Reviewer Committee

Head: *Prof. Dr. Ferenc Fülöp D.Sc.* academician, University of Szeged, Institute of Pharmaceutical Chemistry

Reviewers: *Dr. Gabriella Ujhelyi Ph.D.* honorary associate professor, University of Szeged, Department of Pharmaceutical Technology
Prof. Dr. Romána Zelkó D.Sc., Semmelweis University, University Pharmacy
Department of Pharmacy Administration

Members: *Dr. Zsanett Csoma Ph.D.*, University of Szeged, Department of Dermatology and Allergology

Dr. Zsolt Szakonyi Ph.D., University of Szeged, Institute of Pharmaceutical Chemistry

Szeged

2016

1. INTRODUCTION

Great attention is currently paid to skin care and skin disease prevention. Physical and psychological health are affected to considerable extents by the integrity of the skin. An appropriate skin condition is therefore essential for healthy skin.

From a pharmaceutical industrial standpoint, the skin is a desirable target for drug delivery. The first transdermal drug was approved by the US Food and Drug Administration over 35 years ago. Today, transdermal products represent a growing, multibillion dollar market. The US transdermal drug delivery market approached \$1.2 billion in 2001, \$6.7 billion in 2006 and nearly \$7.9 billion in 2010. By 2015, the global transdermal market is expected to reach \$32 billion (Walter et al., 2015).

Not surprisingly, transdermal drug delivery has received increased attention in recent years thanks to its numerous advantages over the oral and injection routes, such as avoidance of the hepatic “first-pass” metabolism, sustained drug delivery, protection of the gastrointestinal tract from drugs and good patient compliance. Unfortunately, the number of drugs which can be administered by this route is limited. Increase of the number of transdermally applied drugs and the optimization of drug delivery through the human skin are therefore important research opportunities in modern therapy. Ideal transdermal drug delivery systems must be designed to overcome the skin barrier and deliver a therapeutically effective amount of drug through the skin, while skin barrier repair must be facilitated and a good skin condition ensured.

In order to enhance transdermal drug penetration, different methodologies have been investigated and developed. Improvements in physical penetration enhancement technology have led to renewed interest in transdermal drug delivery. Some of these novel advanced transdermal penetration enhancement techniques include iontophoresis, electroporation (EP), ultrasound, microneedles to open up the skin, and more recently the use of transdermal nanocarriers.

More complex drug–device combinations are being developed to overcome the natural barrier function of the skin. From a pharmaceutical technological viewpoint, it is an interesting task to investigate the new transdermal drug delivery techniques, in the interest of the optimization and development of these strategies.

2. EXPERIMENTAL AIMS

The primary aim of my study was to investigate promising transdermal penetration enhancer techniques. To achieve this, the skin barrier structure was examined and different types of novel penetration enhancers were tested alone or in combination from the aspect of the achievement of the effective transdermal drug delivery. The following steps were set.

1. In the first part of my Ph.D. work, the healthy and the diseased skin, especially the unaffected area in the psoriatic skin was studied in order to gain a deeper insight into the highly perturbed functions of the diseased SC, and to help to find the healthy volunteers for the skin measurements.
2. In the second part, different penetration-enhancing strategies were optimized to ensure appropriate transdermal drug delivery. The aims were:
 - to compare the penetration-enhancing effect of a newly developed SE, sucrose myristate (SM), with the generally used sucrose laurate (SL);
 - to investigate the behavior of Transcutol (TR) and Sucrose esters (SEs) applied alone or in combination through the use of a Skin PAMPA artificial membrane system and human skin;
 - to examine the possibility of the spectral analysis of the effects of polyols in sodium lauryl sulfate (SLS)-induced acute irritation;
 - to develop a suitable carrier for the poorly water-soluble, lipophilic phytochemical genistein (GEN);
 - to investigate the transdermal delivery of an LLC-based GEN-containing formulation (LLC-GEN) alone or in combination with EP; and
 - to test the recovery effect of LLC-GEN application and its combination with EP on murine melanoma lesions.

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Sucrose esters and/or Transcutol-containing hydrogels

A chemical penetration enhancer (CPE)-free hydrogel (Control gel) was prepared by the following procedure. 5 wt.% Ibuprofen (IBU, Sigma-Aldrich, St Louis, MO, USA) was dissolved in polyethylene glycol 400 (20 wt.%) (Hungaropharma Ltd., Budapest, Hungary) and this solution was added to a 3 wt.% Carbopol 971 (Lubrizol Corporation/Azelis,

Budapest, Hungary) hydrogel prepared with distilled water. The pH was adjusted to 7.0 by adding trolamine (7 wt.%) (Hungaropharma Ltd., Budapest, Hungary).

Two SEs, SL and SM, were purchased from Mitsubishi-Kagaku Foods Corporation, Tokyo, Japan. To compare the two SEs, 2.64 wt.% of each was incorporated individually in Carbopol 971-based hydrogel (SL gel and SM gel). 2.64 wt.% was chosen because this gave the maximum SE concentration-containing hydrogel which did not require a centrifugation process to remove the bubbles. Similar compositions were prepared by using 10 wt.% TR (Gattefossé and Lubrizol Corporation/Azelis, Budapest, Hungary) (TR gel). 2.64 wt.% SE + 10% TR-containing gels were prepared too (SL + TR and SM + TR gel).

Different concentrations of SE-containing gels were also prepared by a similar method (SL 1; 2; 4; 6; 8; or 10 wt.% gel, SM 0.25; 0.5; 1; 2; 4; 6; or 10 wt.% gel) to investigate the *in vitro* diffusion influenced by the CPE contents.

3.1.2. Genistein-containing lyotropic liquid crystal formulation

GEN was purchased from Extrasynthèse (Genay, France; purity > 95%), and the nonionic surfactant Cremophor RH 40 (CRH40; Polyoxyl 40 Hydrogenated Castor Oil USP/NF) from BASF (Ludwigshafen, Germany). The aqueous phase of the systems was distilled water, and the oil phase was isopropyl myristate (IPM; Merck Kft., Budapest, Hungary).

A GEN-free LLC was prepared by the following procedure. The oil–surfactant mixture (oil:surfactant ratio = 2:1) was homogenized with a magnetic stirrer at room temperature. 10 wt.% of water was then added in small amounts to this mixture during stirring. A similar composition was prepared by using 3 wt.% GEN incorporated in the oil–surfactant mixture with a magnetic stirrer.

3.2. Methods

3.2.1. Drug diffusion and penetration investigations

3.2.1.1. Franz diffusion cell method

Membrane diffusion and permeability studies of active pharmaceutical ingredients (APIs) were carried out with a vertical Franz diffusion cell system (Hanson Microette™ Topical & Transdermal Diffusion Cell System, Hanson Research Co., Chatsworth, CA, USA). A stirring rate of 450 rpm was used. The receptor medium temperature was maintained at 37 ± 0.5 °C throughout the experiments to support the physiological skin temperature (32 ± 0.5 °C) on the membrane in the Franz cell (Mura et al., 2009). The donor phase was 0.30 g of sample,

which was placed on a cellulose acetate membrane (Porafil, Machenerey-Nagel, Düren, Germany, and Pall Life Sciences, Washington, NY, USA) itself (*in vitro*), or in the case of *ex ovo* and *ex vivo* measurements the chorioallantoic membrane (CAM) and the epidermis were supported with the Porafil membrane filter. Samples of 0.8 ml were taken from the acceptor phase by the autosampler (Hanson Microette Autosampling System, Hanson Research Co., Chatsworth, CA, USA) and replaced with fresh receiving medium. Five parallel measurements were made of the amount of drug that penetrated over a time period. The absorbance of the API content was measured with a Unicam Helios α Thermospectronic UV-spectrophotometer v4.55 (Unicam, Thermo Fisher Scientific, Waltham, MA, USA).

3.2.1.2. Skin PAMPA method

The Skin PAMPA model involves recently published artificial membrane-based technology for the fast prediction of skin penetration. The method was reported previously (Vizserálek et al., 2015).

3.2.1.3. Preparation of chorioallantoic membrane

The CAM for the *ex ovo* experiment, was provided by Victor Babes University of Medicine and Pharmacy, Timisoara, Romania. The method of its preparation was described previously (Balázs et al., 2015).

3.2.1.4. Preparation of heat-separated epidermis

Excised human skin from patients who had undergone abdominal plastic surgery was used for the skin permeation studies. The epidermis was separated from the underlying dermis using the heat-separation technique. After excision, the subcutaneous fatty tissue was removed and individual portions were immersed in water bath at 60 °C for 90-120 s and the epidermis was gently removed from the underlying dermis using forceps.

3.2.2. Skin hydration and transepidermal waterloss (TEWL) measurements

The hydrogels were tested on healthy volunteers *in vivo*. The hydration state of the skin before and after treatment was recorded by using Corneometer® CM 825 (Courage and Khazaka Electronic GmbH, Cologne, Germany). The barrier function of the skin was detected by measuring TransEpidermal Water Loss (TEWL) with Tewameter® TM 300 (Courage and Khazaka Electronic GmbH, Cologne, Germany)

3.2.3. Polarization microscopic examinations

The structures of the LLC samples were examined with a polarization microscope (LEICA Q500 MC Image Analyzer System) at room temperature. The magnification was 20x.

3.2.4. Rheological investigations

The rheological profiles of the samples were studied with a PaarPhysica MCR101 rheometer (Anton Paar GmbH, Graz, Austria). The measuring device was of plate–plate type (diameter 25 mm, gap distance 0.2 mm).

3.2.5. Electroporation parameters

The Mezo forte Duo EP device (Serial Number Mez 120905-D) produced by Dr Derm Equipment Ltd. (Budapest, Hungary) was provided by the Derm Clinic of Anti-Aging Dermatology, Aesthetic Laser and Plastic Surgery (Budapest, Hungary). The device operates on the basis of a pulsed electromagnetic field. The polypropylene-covered treating handpiece contains a 25 mm diameter plate electrode in direct contact with the treated surface. Modulation was achieved with high-voltage pulses with a voltage pulse duration of 5 ms, followed by a 20 ms break.

3.2.6. Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy measurements

ATR-FTIR spectra were recorded with an Avatar 330 FT-IR spectrometer (Thermo Nicolet, Waltham, MA, USA) equipped with a horizontal ATR crystal (ZnSe, 45°), between 4000 and 400 cm^{-1} , at an optical resolution of 4 cm^{-1} . 128 scans or in the case of investigations of glycerol (GLY) and xylitol (XYL), 64 scans were co-added and all spectral manipulations were performed with the Thermo Scientific's GRAMS/AI Suite software. No ATR correction was performed.

For the investigation of *human psoriatic skin*, the components of the amide-I band and their relative intensities were estimated semiquantitatively in the 1695–1600 cm^{-1} region of the FTIR spectra by a curve-fitting algorithm, using Gaussian–Lorentzian mixing functions. The best fits were found by an iterative process minimizing the standard error.

For *animal experiments*, in order to obtain a reference spectrum of the API, KBr pellets containing 0.5 mg of SLS or GEN were prepared and used. The spectra of treated and untreated samples were also recorded. Each average layer spectrum of the treated SC was corrected with the corresponding average layer spectrum of the untreated SC. To ensure that no absorbances from the skin itself remained and interfered with the results, the spectra of

untreated control skin samples were subtracted from the spectra of water-treated control skin samples.

3.2.7. Raman measurements

Raman spectra were acquired with a Dispersive Raman Microscope (Thermo Fisher Scientific Inc., Waltham, MA, USA) equipped with a CCD camera and a diode laser operating at 780 nm. The spectra of CRH40, IPM and GEN were collected with an exposure time of 6 s, with 48 scanning and cosmic ray and fluorescence corrections. Raman measurements were carried out with a laser power of 12 mW at a slit width of 25 μm . The localization of GEN in the skin samples was investigated by confocal Raman mapping. For mapping, the microtomed skin samples as depth sections were rotated by 90° and placed on an aluminum surface with the SC toward the top of the plate.

3.2.8. Murine melanoma investigations

Animal studies were conducted on 7–8 week old C57BL/6J female mice. The number of mice involved in the study was 30, divided equally into 6 groups of 5 animals as follows:

Group A: blank group

Group B: mice inoculated with B164A5 cells and otherwise not treated

Group C: mice inoculated with B164A5 cells and treated with LLCs without GEN

Group D: mice inoculated with B164A5 cells and treated with LLCs with 3 wt.% GEN

Group E: mice inoculated with B164A5 cells and treated with LLCs without GEN and EP at 900 V for 6 min

Group F: mice inoculated with B164A5 cells and treated with LLCs with 3 wt.% GEN and EP at 900 V for 6 min

On day 0 of the experiment, the mice in groups B–F received a subcutaneous (s.c.) inoculation of 0.1 ml containing 1×10^6 cells/mouse into the depilated lateral abdomen. For the mice in groups C–F, treatment with 2 ml of a 3 wt.% LLC-GEN formulation was administered from day 2 post-inoculation, using 6 min of high voltage EP or the classical application as described above. Tumor growth was measured daily in millimeters, using calipers, and the tumor volume was estimated by the formula: length x width²/2.

4. RESULTS AND DISCUSSION

4.1. Investigation of the unaffected skin area in the psoriatic skin

In the SC, greater flexibility is achieved through loose packing of the keratin filaments and a lower number of disulfide crosslinks. It was observed that the overall intensities of the

amide-I bands were lower for the psoriatic SC than for the healthy SC, but the peak at 1660 cm^{-1} exhibited the most pronounced alteration, as shown in **Fig. 1**.

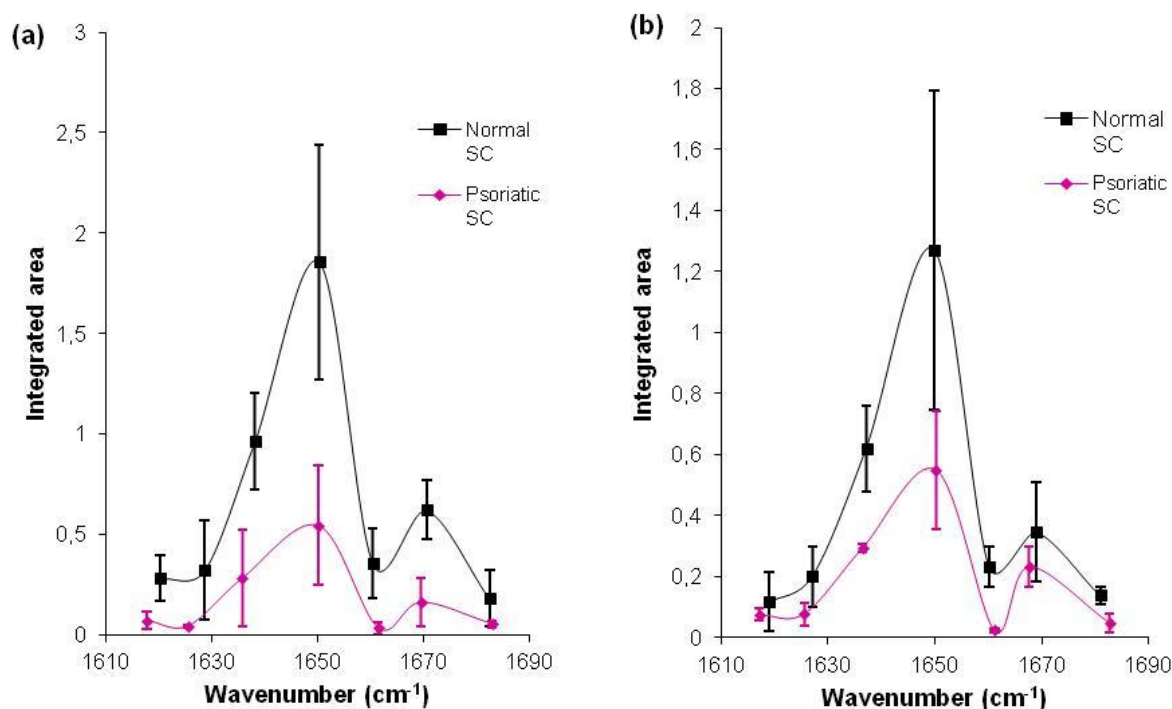


Figure 1. Areas of the fitted components of the amide-I band in the cases of normal and psoriatic SC: (a) man; (b) woman.

The areas of all the amide-I band components are less in the psoriatic group, but the decrease is especially marked in the cases of the bands at 1630 and at 1660 cm^{-1} (**Fig. 1**). The former is in the middle of the region characteristic of the β -sheet structure, and the latter reflects the turn structure. The fall in the intensity of the latter suggests the loss of those parts of the protein structure even in the unharmed psoriatic SC which provide the protein with flexibility.

4.2. Examination of transdermal penetration enhancers

4.2.1. Study of Sucrose esters and/or Transcutol-containing hydrogels

4.2.1.1. Drug diffusion and penetration measurements

To examine the effects of the CPE concentrations on the *in vitro* drug diffusion, we carried out *in vitro* tests with both SE gels in different concentrations. It can be seen that from 2 wt.% SL content the amount of IBU diffused after 24 h were better than in the case of the CPE-free hydrogel. We observed a maximum curve and the best penetration of IBU was strengthened by the 6 wt.% SL gel (**Fig. 2**).

The studies with SM revealed the same tendency. A maximum curve was again observed, but this curve reached the maximum at much lower SE concentration, 0.5 wt.% (Fig. 2).

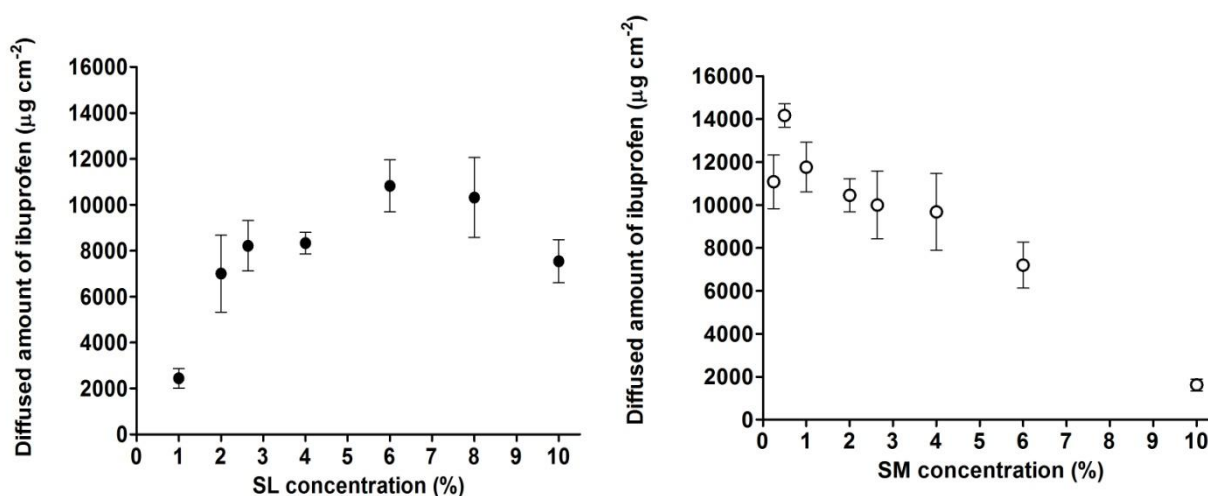


Figure 2. The amount of IBU diffused from different SL and SM-containing hydrogels after 24 h.

Table 1 presents the parameters of IBU penetration through excised human epidermis. The SE gels increased the penetration of IBU through the skin appreciably as compared with the CPE-free Control gel, but the TR gel did not enhance the IBU penetration. TR is thought to give rise to its penetration-enhancing function by increasing the solubility of the applied drug. However, TR has also been observed to display an accumulation property by increasing the skin accumulation of topically applied drugs without a concomitant increase in transdermal penetration. The *ex vivo* drug release behavior data indicated that SL was not a better penetration enhancer for IBU than SM (**Table 1**).

Table 1. Parameters of IBU penetration through the human epidermis after 16 h.

Formulation	Q (µg cm ⁻²)	J (µg cm ⁻² h ⁻¹)	EF
Control gel	463.95±110.50	42.72	-
TR gel	193.36±87.13	14.23	0.33
SL gel	866.79±320.42	67.93	1.59
SM gel	889.22±348.09	54.28	1.27
SL + TR gel	825.08±384.67	54.38	1.27
SM + TR gel	1527.19±764.62	91.59	2.14

The SM + TR gel exhibited the best penetration parameters (**Table 1**). The SL + TR gel resulted in a lower penetration than that with the SL gel, but the difference was not

statistically significant. A possible reason for this phenomenon could be that the TR depot effect predominated in the case of the SL + TR gel, because SL is not able to cause so much lipid disruption. In contrast with SL, SM may cause a greater extent of lipid alteration through its higher carbon chain and this prevents the TR depot effect. Furthermore, SM in combination with TR proved to penetrate the skin well, and there was a marked increase in IBU release from the SM + TR gel as compared with the Control gel.

Table 2 presents the penetration data on the different transdermal formulations tested with the Skin PAMPA system. Lower standard deviation and higher penetration data are observed with the PAMPA model than with the Franz cell method, which may be caused by the differences in the membrane structure in the two procedures. However, the penetration profiles obtained with the Skin PAMPA and *ex vivo* Franz cell methods were in good agreement in most cases.

Table 2. Parameters of IBU penetration through the Skin PAMPA model after 16 h.

Formulation	Q ($\mu\text{g cm}^{-2}$)	J ($\mu\text{g cm}^{-2} \text{h}^{-1}$)	EF
Control gel	5204.95±311.23	432.35	-
TR gel	4941.95±966.97	329.95	0.76
SL gel	5642.38±383.43	517.06	1.20
SM gel	5812.12±150.72	512.81	1.19
SL + TR gel	5616.67±208.33	483.72	1.12
SM + TR gel	6062.98±121.23	563.1	1.30

4.2.1.2. *In vivo* skin tests

The penetration of a drug into the deeper layers can be enhanced through a well-hydrated skin, too. During moisturizing, the intercellular space between the corneocytes increases due to the swelling of the SC. Every investigated sample increased the hydrated state of the skin and ensured a good, lasting moisturizing effect.

4.2.2. Spectroscopic investigations of the effects of polyols in sodium lauryl sulfate-induced acute irritation

The penetration of the dermal preparation components emerge as viewpoint from pharmaceutical technology development of dermal formulations. The penetration characteristics of SLS, as frequently used components, in the presence of the polyols were therefore studied with combined tape stripping and ATR-FTIR spectroscopy. It was found that both lower concentration polyol treatments modified the SLS penetration, but a

significant decrease in the amount of SLS in the deeper layers was observed when higher polyol treatments were applied (**Table 3**).

Table 3. Intensities in the upper and lower layers of the treated SC (relative absorbance of SLS at 1220 cm⁻¹). Median values (M) with 25th and 75th percentiles.

Number of layers	Group 2 (SLS 5 %)		Group 3 (SLS + GLY 5 %)		Group 4 (SLS + XYL 8.26 %)		Group 5 (SLS + GLY 10 %)		Group 6 (SLS + XYL 16.52 %)	
	M	25p; 75p	M	25p; 75p	M	25p; 75p	M	25p; 75p	M	25p; 75p
1-4	0.0226	0.0137 0.0264	0.0309	0.0187; 0.0518	0.0318	0.0181; 0.0468	0.0319	0.0161; 0.0532	0.0196	0.0134; 0.0360
5-8	0.0234	0.0140 0.0433	0.0223	0.0122; 0.0300	0.0241	0.0149; 0.0454	0.00601 *	0.00143; 0.0134	0.000 *	0.000; 0.0133

4.2.3. Investigations of Genistein-containing lyotropic liquid crystal

4.2.3.1. Polarization microscopic examinations

In the development of the dermal delivery, we prepared an LLC formulation which is capable of suspending and partly dissolving GEN in a concentration of 3 wt.%. A polarization microscopic picture of the developed LLC structure reveals a lamellar LLC pattern with a characteristic ribbon structure in polarized light.

4.2.3.2. Rheological investigations

The characteristics of the LLC system include the frequency-dependent storage and loss moduli. In the investigated frequency range, the blank LLC system is more elastic than viscous. The solubilization of GEN in the LLC system led to a consistency increase.

4.2.3.3. Drug diffusion and penetration measurements

The membrane diffusion investigations indicated that good drug release and diffusion was attained through the synthetic membrane *in vitro* (**Fig. 3a**). Somewhat less drug was observed after 24 h (491.26±15.55 µg cm⁻²) in the case of *ex ovo* penetration (**Fig. 3b**). In the *ex vivo* investigations which revealed not only the amount of drug that diffused through the skin, but also the interaction with the skin and the incidental reservoir function of the SC, only a small amount of drug penetrated through the human epidermis, but the penetration profile was similar to that for *ex ovo* penetration and sustained drug release was achieved (**Fig. 3c**).

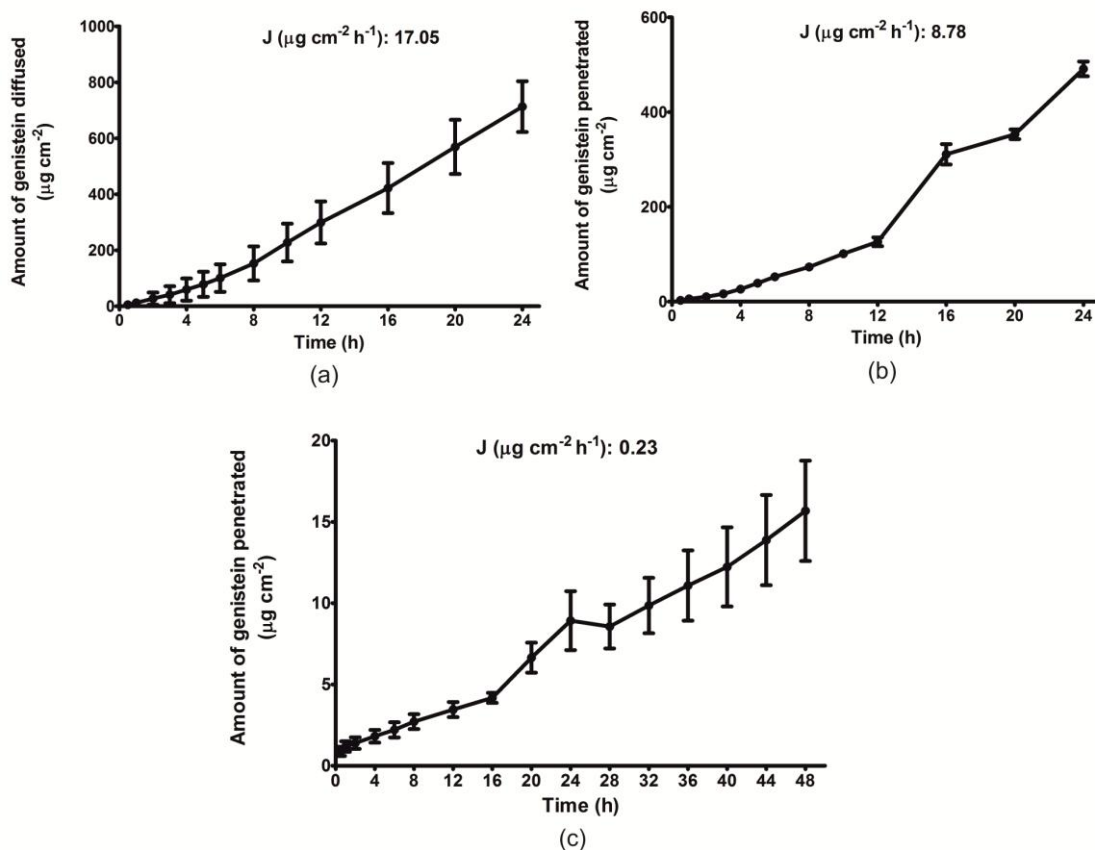


Figure 3. Amount of GEN diffused *in vitro* (a), or penetrated *ex ovo* (b) or *ex vivo* (c).

4.2.3.4. ATR-FTIR spectroscopy

The results showed that the traditionally used LLC-GEN was not effective alone. The LLC-GEN formulation was therefore combined with an active penetration-enhancing method, EP. Different treatment times and voltages were used. The penetration profile of GEN within the different layers of the SC was established with a combination of tape stripping and ATR-FTIR spectroscopy.

It was found that EP treatment at 700 V for 1 min did not enhance the GEN transdermal delivery relative to the conventional treatment (**Fig. 4**). Slightly deeper GEN penetration was ensured by EP treatment at 900 V for 1 min, but the total amount of GEN did not exceed that for conventional treatment with LLC-GEN. However, EP treatment at 700 V for 2 min resulted in a deeper penetration of a 2-fold amount of the drug than without EP treatment (revealed at both characteristic bonds of GEN). The best penetration profile of GEN was obtained on EP treatment at 900 V for 2 min, when the total amount of the drug was 3-fold greater as compared with conventional treatment at both assigned bonds ($p < 0.001^{***}$ and $p < 0.05^*$).

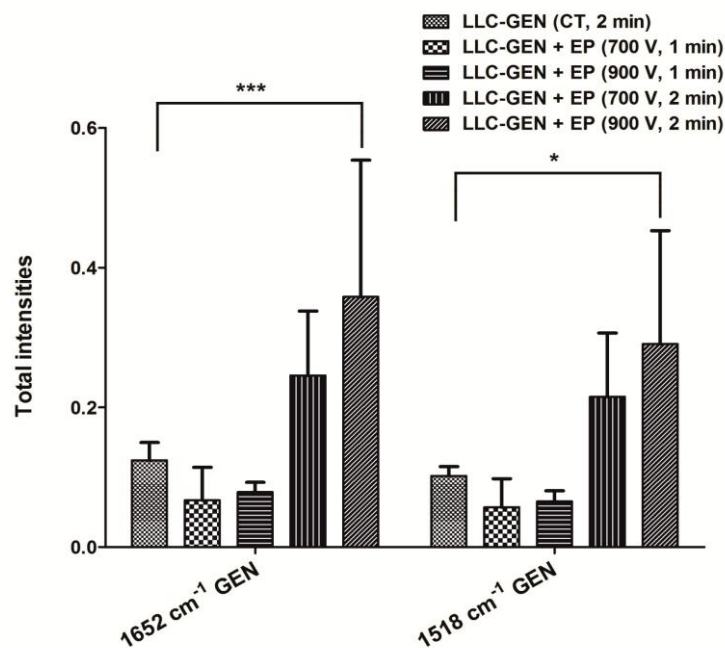


Figure 4. Total intensities of GEN at 1652 cm^{-1} and at 1518 cm^{-1} . Conventional treatment (CT) was used as a control. $p < 0.001$ *** and $p < 0.05$ * vs. EP-treated group.

4.2.3.5. Raman spectroscopy

Mouse skin treated with LLC-GEN combined with EP was examined by Raman spectroscopy. The deeper skin penetration of LLC-GEN containing the ingredients CRH40 and IPM was studied in Raman scattering experiments. Raman chemical mapping was employed to confirm the localization of GEN at various depths of the skin specimens. Since ATR-FTIR demonstrated that EP treatment at 900 V ensured deeper penetration than that at 700 V, EP treatments at 900 V for 1 and 2 min were chosen for comparison with conventional treatment by Raman spectroscopy.

The presence of bands due to GEN and the LLC components in the upper layer of the skin (0–1000 μm) clearly indicates a good distribution following conventional treatment of the LLC-GEN composition (**Fig. 5a**). After the 900 V process for 1 min, GEN was accumulated in the middle section of the dermis (**Fig. 5b**). The penetration of GEN into the dermis was slowed by the dermis being a dense network of collagen fibers. The GEN and LLC component contents of the skin specimen (to a depth of 1300 μm) were relatively low after the 900 V EP process for 2 min (**Fig. 5c**), indicating that the GEN penetrated into the deep layer of the dermis. Although the difference between the EP processes for 1 and 2 min may seem minor, the slower diffusion of GEN may be presumed because of the enrichment of the LLC components (as vehicles of GEN) in the 1000–1300 μm deep section. In the absence

of the EP process, the epidermal and upper dermal regions serve as a tight barrier, impeding deep GEN delivery. Thus, slow diffusion of the GEN took place.

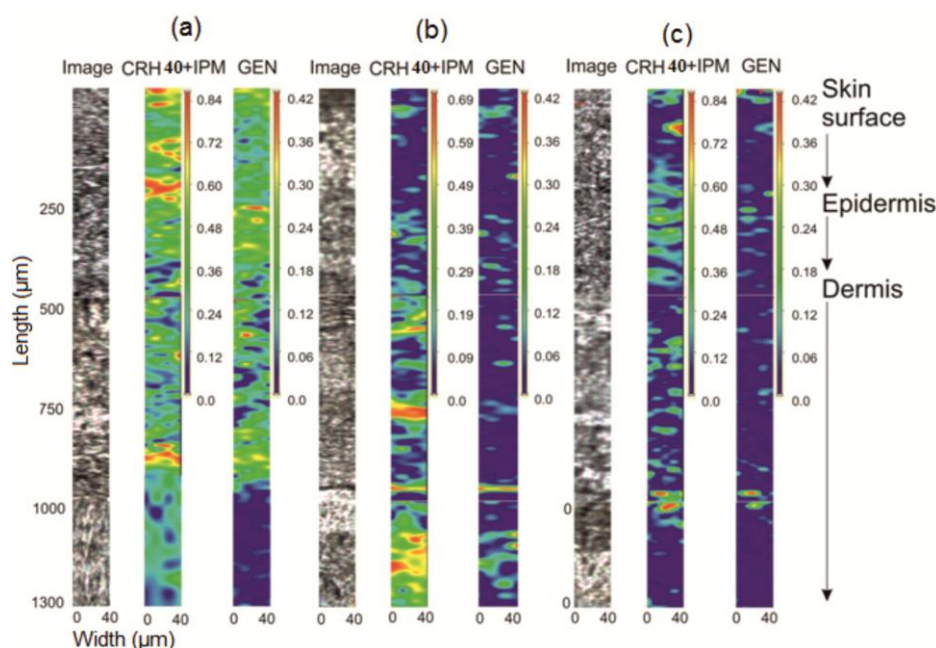


Figure 5. Qualitative Raman maps of GEN distribution in animal skin specimens following different EP protocols (at 10-fold magnification). Conventionally treated skin as control (a), skin treated with EP at 900 V for 1 min (b) and skin treated with EP at 900 V for 2 min (c). Color coding of content: red > yellow > green > blue.

4.2.3.6. Effect of electroporation treatment on murine melanoma

In each of the inoculated mice, the volume of the tumor was observed to be increased, to an extent directly proportional to the number of days of the examination. Tumors appeared on day 8 post-inoculation in both the treated and the untreated groups, with the exception of the mice in group F; in these mice, which were inoculated with B164A5 cells and treated with LLC-GEN and EP for 6 min at high voltage, the tumors appeared on day 10 post-inoculation. Comparison of the curves corresponding to the different treatment approaches reveals that the LLC-GEN formulation decreased the tumor volume, but following EP of this formulation, the results were even better. **Fig. 6.**

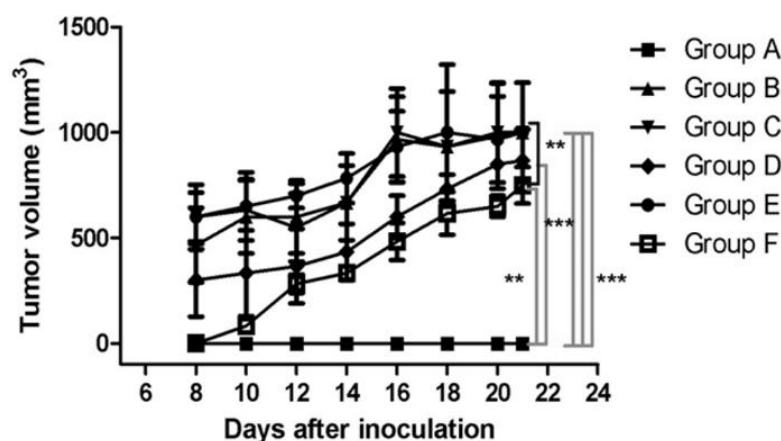


Figure 6. Tumor volumes (mm^3) in the different experimental groups on day 21 of the experiment.

5. SUMMARY

The main aim of my research work was to achieve a better understanding of the SC structure and its barrier function against drug penetration. I compared the unharmed SC in untreated psoriatic patients with that in the healthy control group. A further goal was to investigate different methods of increasing the drug penetration through the skin.

1. Using the components of the amide-I band, intact psoriatic skin was investigated to determine the changes in the secondary structure of the proteins by combining tape stripping and ATR-FTIR spectroscopy. FSD of the amide-I band was followed by curve-fitting to generate the underlying components. Integration of band areas provided an estimate of the secondary structure.

To summarize this part of my Ph.D. work, new results were achieved:

- The protein structure is changed on the unaffected area in psoriatic skin disease.
- The results indicated decreases in all amide-I band components, the peak at 1660 cm^{-1} revealing the most dramatic change. This peak is characteristic of the turn structure in the protein chain. The decrease is marked in the case of the β -sheet structure at 1630 cm^{-1} too.
- The combination of tape stripping and ATR-FTIR spectroscopy could serve as an appropriate, rapid, simple noninvasive method for the study of psoriasis and give the possibility to follow up the efficiency of treatment.

2. In the second part of my Ph.D. work, different types of transdermal drug delivery techniques were tested in order to increase the drug penetration through the skin.

2.1. IBU-containing hydrogels were used with SEs and TR as biocompatible penetration enhancers. The effects of the SE concentrations were examined through a synthetic membrane (*in vitro*) by the Franz cell method and the effects of SE hydrogel formulations on the skin

were evaluated by noninvasive *in vivo* tests. Furthermore, the behavior of these promising CPEs was tested through the use of two different skin test systems.

- The SE concentration influenced the *in vitro* diffusion process markedly. SL showed the highest penetration effect at 6 wt.% concentration, while SM presented the best enhancing effect at 0.5 wt.%. It is not useful to use these SEs at higher concentrations as CPEs.
- The *ex vivo* and Skin PAMPA drug penetration results revealed that TR as cosolvent with SM significantly improved the penetration of the surfactant into the skin and promoted attainment of the maximum penetration effect. SM + TR could be a potential CPE combination for IBU in a dermally used hydrogel.
- Skin PAMPA can serve as an effective and quick prediction model for the monitoring of CPEs and of drug penetration through the skin. The findings with the new artificial PAMPA membrane correlated closely with those with an *ex vivo* human skin membrane.
- The *in vivo* results demonstrated that the incorporation of SEs (and especially SM) into a hydrogel extends and increases the hydration phenomenon. SM significantly improved the moisturizing effect of the hydrogel vehicle.

2.2. Penetration of SLS was investigated combined with polyols. It was concluded from ATR-FTIR spectroscopy that the use of GLY and XYL can decrease SLS penetration. Different concentrations of GLY and XYL were studied and higher polyol treatments decreased the penetration of SLS to the deeper layers of the SC.

2.3. A lamellar LLC was prepared in order to increase the solubility of the lipophilic phytochemical GEN. In order to verify the efficacy of this formulation, membrane diffusion and penetration studies were carried out with a Franz diffusion cell, through a synthetic membrane *in vitro*, a CAM *ex ovo*, and *ex vivo* excised human epidermis. Thereafter, LLC-GEN was combined with EP to enhance the transdermal drug delivery.

- The synergistic effect of EP was verified by *in vivo* ATR-FTIR and *ex vivo* Raman spectroscopy on hairless mouse skin. The penetration through the skin was ensured by the application of LLC-GEN alone. However, combination of the LLC nanocarrier system with EP resulted in rapid and more effective transdermal drug transport than that with the LLC-GEN formulation alone.
- It was confirmed by murine melanoma investigations that EP could be a good method for the delivery of GEN because it delayed the appearance of the tumors and ensured the drug penetration to the deeper layers of the skin.

PUBLICATIONS RELATED TO THE SUBJECT OF THESIS

- I. **Boglárka Balázs**, Gabriella Farkas, Ottó Berkesi, Rolland Gyulai, Szilvia Berkó, Mária Budai-Szűcs, Piroska Szabó-Révész, Lajos Kemény, Erzsébet Csányi: Protein structure is changed in psoriatic skin on the unaffected region – imaging possibility with ATR-FTIR spectroscopy. *MICROCHEMICAL JOURNAL* 117. 183–186. (2014)
IF: 2.746 Citations: 3
- II. Corina Danciu, Marius Biriş, **Boglárka Balázs**, Erzsébet Csányi, Ioana Zinuca Pavel, Georgeta Pop, Codruta Soica, Lavinia Ceuta, Lucian Nita, Claudiu Morgovan, Dana Stoian: Pro-apoptotic Effect of Soy Total Extract Incorporated in Lyotropic Liquid Crystals Formulation, *REVISTA DE CHIMIE* 66. 1038–1041. (2015)
IF: 0.810
- III. Corina Danciu, Szilvia Berkó, Gábor Varju, **Boglárka Balázs**, Lajos Kemény, István Balázs Németh, Andreea Cioca, Alexandra Petruş, Cristina Dehelean, Citu Ioan Cosmin, Elena Amaricai, Claudia Crina Toma: The Effect of Electroporation of a Lyotropic Liquid Crystal Genistein-Based Formulation in the Recovery of Murine Melanoma Lesions, *INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES* 16. 15425–15441. (2015)
IF: 2.862
- IV. **Boglárka Balázs**, Eszter Csizmazia, Szilvia Berkó, Mária Budai-Szűcs, Piroska Szabó-Révész, Erzsébet Csányi: New approach of sucrose myristate as a promising penetration enhancer in dermal preparations, *TENSIDE SURFACTANTS DETERGENTS* 52. 375–379. (2015)
IF: 0.739
- V. **Boglárka Balázs**, Péter Sipos, Corina Danciu, Stefana Avram, Codruta Soica, Cristina Dehelean, Gábor Varju, Gábor Erős, Mária Budai-Szűcs, Szilvia Berkó, Erzsébet Csányi: An ATR-FTIR and Raman spectroscopic investigation of the electroporation-mediated transdermal delivery of a nanocarrier system containing an antitumour drug, *BIOMEDICAL OPTICS EXPRESS* 7. 67–78. (2016)
IF: 3.648

- VI. **Boglárka Balázs**, Gábor Vizserálek, Szilvia Berkó, Mária Budai-Szűcs, András Kelemen, Bálint Sinkó, Krisztina Takács-Novák, Piroska Szabó-Révész, Erzsébet Csányi: Investigation of the efficacy of transdermal penetration enhancers through the use of human skin and a skin mimic artificial membrane, *JOURNAL OF PHARMACEUTICAL SCIENCES* 105. 1134–1140. (2016)
IF: 2.590

PRESENTATIONS RELATED TO THE SUBJECT OF THESIS

- I. Corina Danciu, Ciurlea Sorina, Erzsébet Csányi, **Boglárka Balázs**, Avram Ștefana, Camelia Peev, Dehelean Cristina: A new approach in the recovery of murine melanoma lesions: electroporation of lyotropic liquid crystals genistein based formulation, 5th BBBB International Conference, Athen, Greece, 26-28. September 2013. (poster)
- II. **Balázs Boglárka**: A pszoriázisos bőr fehérje struktúrájának újszerű vizsgálati lehetősége, XX. Szent-Györgyi Napok, Szeged, 2013. november 15. (verbal presentation)
- III. **Balázs Boglárka**, Csizmazia Eszter, Berkó Szilvia, Szabóné Révész Piroska, Csányi Erzsébet: Szacharóz-zsír savészterek penetrációfokozó hatásának vizsgálata, XV. Congressus Pharmaceuticus Hungaricus, Budapest, 2014. április 10-12. (poster)
- IV. **Boglárka Balázs**, Eszter Csizmazia, Szilvia Berkó, Piroska Szabó-Révész, Erzsébet Csányi: Penetration enhancer effect of sucrose esters, Skin Forum 14th Annual Meeting, Prague, Czech Republic, 4-5. September 2014. (poster)
- V. **Boglárka Balázs**, Szilvia Berkó, Mária Budai-Szűcs, Piroska Szabó-Révész, Erzsébet Csányi: Drug diffusion and permeation studies of Genistein containing Lyotropic liquid crystals, 5th International Conference and Exhibition on Pharmaceutics & Novel Drug Delivery Systems, Dubai, UAE, 16-18. March 2015 (poster)
- VI. **Boglárka Balázs**, Mónika Bakonyi, Eszter Csizmazia, Szilvia Berkó, Mária Budai-Szűcs, Piroska Szabó-Révész, Erzsébet Csányi: Non-invasive in vivo comparison of penetration enhancers containing hydrogel formulations, 1st European Conference on Pharmaceutics - Drug Delivery, Reims, France, 13-14. April 2015. (poster)

- VII. **Balázs Boglárka**: Szacharóz-zsírsvészterek penetrációfokozó hatásának vizsgálata, Richter Gedeon Centenáriumi Alapítvány előadótülés, Budapest, 2015. március 05. (verbal presentation)
- VIII. Csupor Dezső, Jedlinszki Nikoletta, **Balázs Boglárka**, Csányi Erzsébet: Veszélyes-e a nadálytő? Egy farmakokinetikai vizsgálat tanulságai, XIV. Magyar Gyógynövény Konferencia, Pannonhalma, Magyarország, 2015. május 29-30. (poster)
- IX. **Boglárka Balázs**, Attila Léber, Szilvia Berkó, Mária Budai-Szűcs, Piroska Szabó-Révész, Erzsébet Csányi: In vivo investigation of newly developed microemulsion and lyotropic liquid crystal systems, 4th International Conference and Expo on Cosmetology & Trichology, Philadelphia, USA, 22-24. June 2015. (poster)
- X. Nikoletta Jedlinszki, Anikó Dobó Beretka, **Boglárka Balázs**, Dezső Csupor: Absorption of lycopsamine from a comfrey ointment through human skin, 63rd International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA2015), Budapest, Hungary, 23-27. August 2015. (poster)
- XI. Léber Attila, **Balázs Boglárka**, Berkó Szilvia, Csányi Erzsébet: Új típusú nanohordozó rendszerek bőrre gyakorolt hatásának tanulmányozása non-invazív NIR spektroszkópiái és *in vivo* vizsgálati módszerekkel, Gyógyszertechnológiai és Ipari Gyógyszerészeti Konferencia, Siófok, 2015. október 15-17. (poster)
- XII. **Balázs Boglárka**, Berkó Szilvia, Csányi Erzsébet: Elektoporáció, mint a bőrön keresztüli hatóanyag bejuttatás új típusú lehetősége, Gyógyszertechnológiai és Ipari Gyógyszerészeti Konferencia, Siófok, 2015. október 17. (verbal presentation)