University of Szeged  
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Summary of the Ph.D. thesis  

IMPROVED SKIN PENETRATION USING ACTIVE AND PASSIVE ENHANCEMENT METHODS  

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1. INTRODUCTION

The dermal delivery of active agents is an attractive alternative to conventional administration routes, especially when the pathological conditions intended to be treated are located in the skin. This administration route offers lower dosage and lower systematic toxicity with effective local concentration, in addition, it is a non-invasive and patient-friendly method. Furthermore, topically applied drugs avoid hepatic first pass metabolism and the degradation in the gastrointestinal system if they are absorbed.

One of the most compelling medical challenges of this century is the treatment of cancer. Skin cancer has shown increasing incidence over the past decades for various reasons in connection with the increased ultraviolet radiation exposure, environmental and genetic factors. Skin cancer at early stages is localized within the upper layers of skin, thus the dermal application of anticancer agents in the localized region is advantageous for the effective treatment of the tumor, such as for postoperative treatments.

Effective topical local anesthesia is also a challenge nowadays because most of the marketed topical formulations have moderate skin penetration properties, a rapid but short effect. Thus the development of local topical anesthetics with a prolonged release at the site of action is also desperately desired. Both of the above-mentioned therapeutic goals require the penetration of the drugs into the dermal layers of the skin.

However, because of the barrier function of the outermost layer of the skin, the stratum corneum, only small and lipophilic molecules are able to penetrate through the skin by passive diffusion. Numerous technologies have been developed to enhance the penetration of molecules through this barrier based on various strategies: increasing skin permeability, providing a driving force acting on the drug, or the combination of these methods. They can be categorized as passive and active approaches.

The selection of an adequate drug carrier system with proper material attributes and/or the application of active and passive penetration enhancement methods are/is fundamental to reaching the aim of application. With a rationalized, scientific and risk-based initial theoretical development before the technical development, parameters that most strongly influence the final formulation can be discovered and monitored. With the consideration of the Quality by Design approach related to the development of new pharmaceutical formulations, a cost-saving process ensuring a high-quality product taking into account patient expectations, industrial and regulatory aspects can be achieved.
2. EXPERIMENTAL AIMS

The aim of my Ph.D. work was to investigate promising dermal therapies, including novel drug delivery systems, active and passive penetration enhancement methods, and the opportunity to model the skin during the formulation development process.

1. In the first part of my work, liposomal skin modeling systems were prepared and investigated. The aim of this part was to evaluate the feasibility of in vitro model systems for predicting the effects of penetration enhancers on the skin. Two modern chemical penetration enhancers, Kolliphor RH40 and Transcutol, were investigated. Their enhancement effect was also studied by ex vivo penetration experiments. The correlation between in vitro and ex vivo results was calculated, thus the applicability of liposomal skin models for evaluating the effect of chemical penetration enhancers was determined.

2. In the second part, a topical betulinic acid-containing anticancer formulation was developed, and its penetration through the skin was followed-up by Raman spectroscopic mapping. The effect of electroporation (EP) on the penetration of betulinic acid (BA) was also determined. The aim was to develop a potential non-invasive, topical therapy for cutaneous melanoma.

3. In the third part, different lidocaine-containing carrier systems were developed and investigated.

The following aims were set:

- to incorporate the salt and base form of lidocaine (LID) in conventional and novel drug delivery systems: hydrogel, oleogel, liposome, nanostructured lipid carrier (NLC) and lyotropic liquid crystal (LLC)
- to characterize the vehicles in terms of:
  - particle size (dynamic light scattering method),
  - zeta-potential (electrophoretic mobility method),
  - encapsulation efficiency (ultracentrifugation, DSC),
  - drug-carrier interactions (FT-IR and Raman spectroscopy);
- to apply the Quality by Design (QbD) method for determining the most critical characteristics influencing the quality of the formulation which have to be examined during the development process;
- to apply differential scanning calorimetry (DSC) as a novel approach for the determination of the entrapment efficiency of liposomes;
• to examine the release and skin penetration of lidocaine incorporated in different carrier systems in vitro and ex vivo by a vertical Franz diffusion cell;

• to investigate the influence of the carrier systems on the physiological skin conditions in vivo (skin hydration and TEWL);

• to follow-up drug penetration into skin cross-section by Raman spectroscopic mapping.

3. MATERIALS AND METHODS

3.1. Preparation of skin modeling liposomes

Stratum corneum lipid liposomes (SCLLs) were prepared by thin film hydration method. Lipid composition was chosen to represent the stratum corneum (SC): ceramides: cholesterol: palmitic acid: cholesterol sulfate= 4: 2.5: 2.5: 1 wt%. The hydration was done with 20 mM HEPES buffer (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) containing 0.8 mM EDTA (Sigma-Aldrich, Saint Louis, Missouri, USA) and 120 mM NaCl (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) or 70 mM calcein dye (Sigma-Aldrich, Saint Louis, Missouri, USA) instead of NaCl in case of efflux measurements at pH 8. The suspensions were sonicated in a bath sonicator at 80°C for 1 h, then annealed for 30 min.

3.2. Preparation of betulinic acid-containing formulation

Betulinic acid (Cayman Chemical Company, Ann Arbor, USA) was partly dissolved in the mixture of 2-propanol (Merck Ltd., Budapest, Hungary), Transcutol, Labrasol (Azelis Hungary Ltd., Budapest, Hungary) and isopropyl myristate (Merck Ltd., Budapest, Hungary). PEG 4000 (Merck Ltd., Budapest, Hungary) was melted and homogenized with this suspension. The ointment was stirred until cooling.

3.3. Preparation of lidocaine-containing formulations

3.3.1. Hydrogel

5 wt% lidocaine hydrochloride (Hungaropharma Ltd., Budapest, Hungary) was dissolved in the mixture of purified water, ethanol and macrogol 400 (Hungaropharma Ltd., Budapest, Hungary), and 3 wt% Methocel K4M (Colorcon, Budapest, Hungary) was added to this solution as the gelling agent.

3.3.2. Oleogel

Mygliol 812N (Sasol GmbH, Hamburg, Germany) and Kolliphor RH40 (as a penetration enhancer) (BASF SE Chemtrade GmbH, Ludwigshafen, Germany) mixture was heated to 60°C and 5 wt% lidocaine base (Hungaropharma Ltd., Budapest, Hungary) was dissolved in this
mixture. 5 wt% Aerosil 200 (Sigma- Aldrich, Saint Louis, Missouri, USA) was added to prepare gels.

3.3.3. Liposome

Lidocaine-containing liposomes were prepared by dry film hydration method. Lidocaine concentration was set to 1-2-3-4-10 wt%. The hydration of the film was done with 1 mL HEPES buffer (20 mM, containing 154 mM NaCl, pH= 7.4) at room temperature, alternating with vortex agitation for 5 min.

3.3.4. LLC

Isopropyl myristate (Merck Ltd., Budapest, Hungary) and Kolliphor RH40 1:4 mixture was heated (60°C) and homogenized with a magnetic stirrer, 5 wt% lidocaine base was dissolved and the water phase was then added to the lipid phase drop by drop under stirring.

3.3.5. NLC

The solid lipid Apifil (PEG-8 Beeswax, Gatelfossé, St. Priest, France) was melted at 80°C, Mygliol 812N and lidocaine base were added. Under the same conditions, the water phase was prepared containing the surfactant (Cremophor RH60, BASF SE Chemtrade GmbH, Ludwigshafen, Germany). Then, the water phase was added to the lipid phase and this pre-emulsion was ultrasonified using a Hielscher UP200S ultrasonic homogenizer (Hielscher Ultrasonics GmbH, Germany). The sample was cooled down in an ice bath to get the NLC dispersion. Then, a gel was formed (at room temperature) with glycerin and Methocel K4M and the NLC dispersion was added to reach a final 5 wt% lidocaine concentration.

3.4. Particle size and zeta potential measurements

Particle size and zeta potential measurements for liposomes and NLC were taken with a Malvern Nano ZS device (Malvern Instruments, Malvern, UK), in standard disposable cuvettes using Malvern’s dip cell. The polydispersity index (PDI) was also evaluated to define the homogeneity of the dispersion.

3.5. Ultracentrifugation

To determine encapsulation efficiency, liposomes were centrifuged in a Beckman Coulter Optima XE-90 Ultracentrifuge (Beckman Coulter Inc., Brea, CA, USA) for 3 h at 35,000 rpm at 4°C. Both the supernatant (“indirect method”) and the pellet (dissolved in 1 mL ethanol “direct method”) were measured with UV spectrometer at 262 nm to determine encapsulation efficiency.
3.6. Differential scanning calorimetry measurements

DSC measurements of lidocaine-containing liposomes were performed using a MicroCal VP-DSC device (MicroCal Inc., Northampton, USA). A heating rate of 1°C min⁻¹ in the 5–80°C range was applied. Origin 7.0 software was used to process measurement data. DSC was also used for the evaluation of encapsulation efficiency as a novel approach: the reduction of phase transition temperature depends on the partitioning between LID and the lipid in the fluid or in the gel phase.

\[ \Delta T_{m}(X) = -\frac{RT^2}{\Delta H}X_d^b \]  

(1)

where \( R \) is the ideal gas constant (1.9858775 cal K⁻¹ mol⁻¹), \( \Delta H \) is the enthalpy of the main phase transition, and \( X_d^b \) is the molar fraction of the drug bounded in the liposome.

3.7. Polarization microscopic analysis

LLC samples were investigated under polarized light microscopy (Leica Q500 MC Image Analyzer System) using a magnification of 200×.

3.8. Fourier transform infrared spectroscopic analysis

The FT-IR spectra of LID-NLC were recorded with an Avatar 330 FT-IR spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) equipped with a horizontal ATR crystal (ZnSe, 45°), between 4000 and 400 cm⁻¹, with 128 scans, at an optical resolution of 4 cm⁻¹.

3.9. Raman spectroscopic measurements of lidocaine-containing formulations

Raman spectra of the formulations were acquired with a Thermo Fisher DXR Dispersive Raman Spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) with a laser operating at 532 nm. Spectra were recorded with an exposure time of 6 s, with 24 scans, including cosmic ray and fluorescence corrections. Measurements were carried out with a laser power of 10 mW at a slit width of 25 μm.

3.10. Calcein efflux and lifetime-based fluorescence leakage assay

To determine the disruption effect brought about by CPEs degree of calcein efflux from SCLLs was examined. The efflux (E) was calculated according to the following equation:

\[ E = \frac{(B_F - B_{F0})(B_F - B_{F0} + Q_{stat}B_E)}{B_{F0}} \]  

(2)

where B is the pre-exponential factor (proportional to concentrations of free and entrapped dye). \( B_{F0} \) corresponds to the free dye at the beginning of the measurement, \( B_F \) to free dye concentration at certain time points and \( B_E \) to the entrapped calcein concentration. \( Q_{stat} \) is the static quenching factor which considers that some entrapped dye might be ‘invisible’ due to dimerization in the ground state. We used 1.2 as \( Q_{stat} \) based on the literature.
Measurements were performed in a Horiba Jobin Yvon (Horiba Ltd., Edison, New Jersey, USA) Fluorolog 3 system equipped with a 467 nm laser diode pulsed at 1 MHz for excitation. The decay curve was recorded at a wavelength of 515 nm (bandwidth 2 nm) for 25 s using time-correlated single photon counting.

3.11. Drug permeability studies in vitro and ex vivo

In vitro diffusion and ex vivo penetration was measured using a vertical Franz diffusion cell system (Hanson Research, Chatsworth, CA, USA). For in vitro measurements mixed cellulose ester membrane (Porafil, Macheneroy-Nagel, Düren, Germany) was applied itself and in case of ex vivo measurements heat separated human epidermis was supported with a Porafil membrane filter. The amount of permeated drug was measured using a Unicam Helios Thermospectronic UV-VIS spectrophotometer (Unicam, Thermo Fisher Scientific Inc., Waltham, MA, USA) in case of lidocaine at 262 nm or high-performance liquid chromatography (HPLC) in case of caffeine.

3.12. Raman mapping

The localization of lidocaine in skin samples was investigated by confocal Raman mapping. Microtomed skin cross-sections were placed on an aluminum surface with the SC toward the top of the plate. Raman spectroscopic measurements were performed with a Thermo Fisher DXR Dispersive Raman Spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). The excitation source was 780 nm laser light, power was 24 mW. Measurements were recorded using a 50x objective. The acquisition time of each spectrum was 12 times 2 s and the spectrograph aperture was adjusted 25 μm slit. Data acquisition and analysis were accomplished using OMNIC™ 8.2 for Dispersive Raman software package (Thermo Fisher Scientific Inc., Waltham, MA, USA).

3.13. Electroporation experiments

Animal studies were conducted on six-month-old female SKH-1 hairless mice. The mice involved in the study were divided into 4 groups as follows:

- Group A: mice treated with dermally applied BA ointment for 30 min (conventional treatment)
- Group B: mice treated with BA ointment and EP at 900 V for 1 min
- Group C: mice treated with BA ointment and EP at 900 V for 2 min
- Group D: mice treated with BA ointment and EP at 900 V for 6 min

A Mezoforte Duo Mez 120905-D instrument (Dr. Derm Equipment Ltd., Budapest, Hungary) was used to generate the electric pulses for the skin electroporation.
3.14. Skin hydration and TEWL measurements
Formulations were tested on 3-4-month-old male SKH-1 hairless mice in vivo. Skin hydration was measured using a Corneometer CM 825 and TEWL was evaluated with Tewameter TM 300, both connected to a Multi Probe Adapter MPA 5 (Courage+Khazaka Electronic GmbH, Germany) before and 30, 90 and 150 min after the treatment.

3.15. Statistical analysis
One-way ANOVA followed by the Bonferroni test was used to determine the statistical differences between the results by using GraphPad Prism software (GraphPad Software Inc., La Jolla, CA, USA). Differences were regarded as significant if p<0.05*, p<0.01** and p<0.001***.

4. RESULTS AND DISCUSSION
4.1. Investigation of SCLL liposomes as potential models for skin
4.1.1. SCLL characteristics: particle size and polydispersity index
The particle size of SCLLs was 135.23 ±0.06 nm and PDI was 0.220±0.02. The particle size of the calcein-loaded SCLL was 137.35±0.07 nm and their PDI was 0.228±0.02.

4.1.2. Calcein efflux and lifetime-based fluorescence leakage assay
The effect of different concentrations of Kolliphor RH40 and Transcutol was tested on the disruption of SCLLs using calcein leakage assay. Both Kolliphor RH40 and Transcutol promoted calcein efflux from SCLLs in a concentration-dependent manner. However, from Transcutol, much higher concentration was needed to achieve the same efflux value (Figure 1).

![Fig. 1. Efflux from SCLLs induced by Kolliphor RH40 (A) and Transcutol (B) as a function of incubation time. The concentrations of the penetration enhancers are indicated in the plot.](image)

We used fluorescence lifetime measurement method to obtain information about the leakage mechanism. The efflux can result mainly from two mechanisms: (i) all-or-none, when only
some vesicles are disrupted (and releasing all entrapped dye) while others remain undamaged or (ii) graded, when all vesicles release a certain fraction of their content. Vesicles incubated with Kolliphor RH40 showed all-or-none leakage mechanism until a certain point around 60% efflux, then it became graded (Figure 2A). Transcutol caused graded leakage of the vesicles (Figure 2B).

![Fig. 2.](image)

**Fig. 2.** Calcein efflux as a function of the entrapped fluorescence lifetime on a reciprocal scale brought on by Kolliphor RH40 (A) and Transcutol (B).

### 4.1.3. Effect of penetration enhancers on *ex vivo* penetration of caffeine

Figure 3 shows the cumulative amounts of caffeine penetrated through heat separated epidermis over 24 hours. It can be stated that the *ex vivo* penetration of caffeine was strongly dependent on the concentration of Kolliphor RH40. The highest concentration of Kolliphor RH40 applied was 8.1% and it caused 21% increase in the penetration of caffeine compared to the blank caffeine solution after 24 h.

![Fig. 3.](image)

**Fig. 3.** The cumulative amount of caffeine penetrated through heat separated epidermis after application of different concentrations of Kolliphor RH40.
4.1.4. The relationship between calcein leakage and ex vivo skin penetration

We attempted to evaluate the correlation between the in vitro SCLL-based data and the ex vivo results gained by conventional penetration experiments. The correlation coefficient of this relationship was 0.915 after 1 h and 0.954 after 2 h (Figure 4). Our results suggest that SCLLs could be promising in vitro approaches for screening the effects and effective concentrations of chemical penetration enhancers as we found a good correlation between SCLL-based experiments and the skin penetration study.

![Graph showing the relationship between calcein leakage and ex vivo skin penetration](image)

**Fig. 4.** The relationship between calcein leakage and increase in the caffeine permeated compared to blank formulation after 1 and 2 hours.

4.2. Examination of the penetration of the betulinic acid-containing formulation

Figure 5 displays qualitative distribution maps of betulinic acid in animal skin specimens after different EP protocols. Without EP, the epidermal and upper dermal regions serve as a tight barrier limiting the diffusion of BA. After 1 min 900 V EP treatment, BA penetration was remarkably deeper and showed better distribution in the whole skin section (Fig. 5B). The BA content of the skin specimen (to a depth of 700 μm) was relatively lower after the 900 V 2 min EP treatment, mostly present in the upper layers of the epidermis and the deeper regions (Fig. 5C). After 6 min 900 V treatment, the drug disappeared from the upper layers and was present in the undermost layers of the dermis, indicating the penetration to deeper regions, and presumably potential to systematic absorption (Fig. 5D).
Fig. 5. Qualitative Raman maps of BA distribution in animal skin specimens following different EP protocols. Conventionally treated skin as control (a), skin treated with EP at 900 V for 1 min (b), for 2 min (c) and for 6 min (d). Colour coding of content: red>yellow>green>blue

4.3. Investigation of the properties and performance of lidocaine-containing formulations

4.3.1. Characterization of the carrier systems

4.3.1.1. Liposome

4.3.1.1.1. Particle size and zeta potential measurements

The particle size of liposomes was in the range of $1844 \pm 562.9$ nm to $4842 \pm 275.57$ nm. The results presented a decrease in vesicle size and a reduction in homogeneity with increasing the amount of the added LID, as indicated by the growth of the PDI. Measurements of zeta-potential values showed that it was barely influenced by the presence of the drug, its value altered around $0$ mV, so a contribution of the drug to the liposomal charge can be excluded.

4.3.1.1.2. DSC

The effect of LID on the thermotropic behavior of DMPC was investigated by DSC as a function of the anesthetic concentration. The main phase transition for DMPC—represented by the peak in $Cp$—is progressively lowered and broadened with increasing concentrations of LID (Figure 6). The position of the main peak is reduced from $Tm=23.85 \degree C$ for DMPC down to about $22.66 \degree C$ with $10$ wt% LID. This suggests a chain disordering effect in the lipid membrane.
4.3.1.2. Encapsulation efficiency

The encapsulation efficiency (EE%) values of the liposomal formulations obtained by different experimental methods are represented by Table 1. With indirect method, we measured notably higher EE % values (9.4-32.2 %) and high standard deviation, while the results of the direct method (3.5-10.1 %) and the DSC measurements (5.4-13.0 %) correlate nicely. Considering these outcomes, we can conclude that the direct method and DSC confirmed each other’s results, while the indirect method suffered from an unknown error source.

<table>
<thead>
<tr>
<th>LID (wt %)</th>
<th>Indirect method</th>
<th>Direct method</th>
<th>DSC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EE%± SD</td>
<td>$K_R$ (mM$^{-1}$)</td>
<td>EE%± SD</td>
</tr>
<tr>
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<td>0.0553</td>
<td>10.1% ± 4.2*</td>
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</tr>
<tr>
<td>3</td>
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<td>0.0637</td>
<td>4.4% ± 1.8***</td>
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<tr>
<td>10</td>
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<td>3.5% ± 3.2</td>
</tr>
</tbody>
</table>

4.3.1.3. LLC

4.3.1.3.1. Polarization Microscopic Examinations

Figure 7 presents a polarized microscopic picture of the developed blank and lidocaine-containing LLC structures, revealing a lamellar LLC pattern with a characteristic „Maltese cross” structure in polarized light.
4.3.1.4. NLC

4.3.1.4.1. Results of particle size and zeta potential analysis

The particle size of the lidocaine-containing NLC system was 87.61±0.55 nm. The calculated PDI was 0.1615±0.01, referring to a monodisperse distribution. The low PDI predicts a satisfying stability of the system. The zeta potential was -11.983±0.31 mV.

4.3.1.4.2. Investigation of lidocaine-carrier system interactions

For the determination of LID-excipient interactions in the NLC formulation at the level of functional groups, we applied FT-IR and Raman spectroscopy. All the characteristic peaks of the drug and the excipients were present in the LID-NLC spectrum and no shifting of existing peaks or creation of new peaks were observable (Figure 8). This indicates that there were only physical interactions between the drug and the excipients, and no chemical interaction took place among them.

![Fig. 7. Polarizing microscopic examination of blank (A) and lidocaine-containing (B) LLC](image)

![Fig. 8. FT-IR (A) and Raman (B) spectra of individual components and NLC compositions. Notes: Apifil (1), Cremophor RH60 (2), Mygliol 812 N (3), Lidocaine (4), Lidocaine-free NLC (5), Lidocaine NLC (6)](image)
4.3.2. Comparative study of dermal local anesthetic formulations based on QbD approach

4.3.2.1. Definition of QTPP and CQAs for a dermal local anesthetic formulation

We aimed to select the best semisolid local anesthetic formulation for dermal use, which can provide effective and prolonged anesthesia. This therapeutic efficacy of the formulations depends on therapeutic indication, route of administration, site of activity, dosage form, release profile, stability and dosage strength. The critical quality attributes were identified as physical attributes, the solubility of API in the drug product, homogeneity of API in the drug product, in vitro drug release, ex vivo drug release, moisturizing effect, TEWL, dosage form type and viscosity.

4.3.2.2. Initial risk assessment

Based on the results of risk assessment in vitro and ex vivo drug release, dosage form type, moisturizing effect and TEWL, with the highest severity score (>300), are the most critical characteristics influencing the quality of the formulation. These are the critical attributes which have to be considered during the development process, thus, these points have been investigated in this research.

4.3.2.3. The results of in vitro release and ex vivo permeation studies

In Figure 9, the plots of the cumulative permeated amounts of LID as a function of time are shown. In vitro, LLC formulation showed a complete release, while conventional hydrogel exhibited a fast onset of diffusion, but it was not completed after 24 h. The oleogel and NLC formulations provided a sustained release.

Through heat separated epidermis, ex vivo, LID permeation from the oleogel exhibited the lowest value. There was no significant difference between the penetration of conventional hydrogel and LLC. Surprisingly, the NLC formulation exhibited the highest penetration, whereas in vitro it was the lowest. This finding could be explained by the special structure of the NLC, which allows the high bioavailability of this formulation.
4.3.2.4. Skin hydration and TEWL measurements

Both hydrogel and LLC increased the hydration slightly after 30 min, but this effect was shortened after 150 min. However, oleogel caused a dramatic decrease of hydration after 30 min. After 150 min, the skin barrier seemed to have recovered partially, and the hydration value was closer to the initial level. NLC resulted in a remarkable increase in skin hydration after 30 min and the hydration maintained a high level also after 150 min (Figure 10A).

NLC caused the slightest increase in TEWL, while conventional hydrogel and oleogel increased id moderately. The TEWL rise was the most significant for LLC, presumably because of the high amount of surfactant incorporated in the formulation (Figure 10B).

![Graph A: Relative hydration changes normalized to levels before treatment (%)](image)

![Graph B: Relative TEWL changes normalized to levels before treatment (%)](image)

**Fig. 10.** Skin hydration (A) and transepidermal water loss (B) changes after exposure to formulations. Values are given as percentage referring to values before treatments vs. conventional hydrogel. p < 0.05*, p < 0.01** and p < 0.001***

4.3.3. Following-up skin penetration of lidocaine-containing formulations *ex vivo*

Figure 11 displays qualitative distribution maps of LID in human skin specimens after the treatment with hydrogel, oleogel, LLC and NLC formulation.

The spatial distribution of lidocaine is quite homogenous in the case of hydrogel and oleogel, the drug is present also in the epidermal and the dermal skin layers but showing lower intensity values. LLC and NLC showed much higher intensity values in the treated skin specimens. The lidocaine concentration at deeper skin layers was substantially increased compared to the surface.
Fig 11. Video images and qualitative Raman maps of LID distribution in human skin specimens after 6 hours following treatment with hydrogel (a), oleogel (b), LLC (c) and NLC (d). Untreated skin is also displayed as a control in all cases. Color coding of content: red>yellow>green>blue.

5. SUMMARY

The aim of my Ph.D. work was to achieve a better understanding of active and passive skin penetration enhancement methods through investigating their effect on the penetration of novel anti-melanoma and local anesthetic formulations.

1. In the first part of my work, the applicability of stratum corneum lipid liposomes was investigated as possible *in vitro* model for investigating the effects of chemical penetration enhancers, such as Kolliphor RH40 and Transcutol.

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

- the concentration dependence of penetration enhancement by Kolliphor RH40 and Transcutol was demonstrated;
- the mechanism of the disruption of the lamellar structure of skin modeling liposomes was revealed;
- *in vitro-ex vivo* correlation was determined, thus the applicability of SCLLs as *in vitro* skin models was proved.
2. In the second part of my Ph.D. work, the effect of EP on the penetration of betulinic acid incorporated in an ointment formulation was determined. The results led to the following conclusions:

- the penetration depth of betulinic acid can be remarkably ameliorated by the use of electroporation;
- the influence of various EP treatment times was proved, since they caused different spatial distributions of the drug in the skin;
- after a 6-min EP treatment, a significant amount of BA reached the undermost layers of the dermis;
- the combination of EP and BA can be a promising opportunity to treat tumors in the deeper skin regions in a non-invasive way.

3. In the third part of my thesis, different lidocaine-containing carrier systems were formulated and their properties and effects were compared using in vitro, ex vivo and in vivo methods. The use of the QbD concept during the development process revealed the critical points that should be investigated. The novelty of this work can be summarized in the following:

- Quality Target Product Profile and Critical Quality Attributes were identified for the development of a dermal local anesthetic formulation, furthermore, an initial risk assessment was done in order to select the control points;
- 3 types of novel nanostructured lidocaine carriers were successfully developed and characterized: liposomes, NLCs and LLCs;
- the encapsulation efficiency of lidocaine-containing liposomes was investigated using DSC. The results were compared with those obtained with ultracentrifugation. This research revealed a less known application field of DSC, as a fast and reliable tool to determine EE%;
- among the investigated carrier systems, the nanostructured lipid carrier has proven to be the most promising vehicle for the topical delivery of lidocaine. It showed the best penetration properties through heat separated epidermis and the highest moisturizing effect, which are the most critical parameters based on the Quality by Design initial risk assessment and evaluation;
- the application of Raman spectroscopy provided the visualization of spatial distribution in the skin for different lidocaine-containing formulations. The results confirmed the raison d'être of developing modern carrier systems, as the nanostructured LLC and NLC showed noticeably better skin penetration compared with conventional hydrogel and oleogel;
- this work also highlighted the ability of Raman spectroscopy as a nondestructive technique for investigating skin distribution and tracking penetration pathways of active agents.
PUBLICATIONS RELATED TO THE SUBJECT OF THE THESIS


   *Acta Pharmaceutica Hungarica* 87 3-12 (2017)

   **IF:** -

II. **Mónika Bakonyi**, Szilvia Berkó, Mária Budai-Szűcs, Anita Kovács, Erzsébet Csányi: DSC for evaluating the encapsulation efficiency of lidocaine-loaded liposomes compared to the ultracentrifugation method.

   *Journal of Thermal Analysis and Calorimetry* 130 (3) 1619-1625 (2017)

   **IF:** 1.953 (2016)

   **Citations:** 1

III. **Mónika Bakonyi**, Szilvia Berkó, Anita Kovács, Mária Budai-Szűcs, Nikolett Kis, Gábor Erős, Ildikó Csóka, Erzsébet Csányi: Application of Quality by Design principles in the development and evaluation of semisolid drug carrier systems for the transdermal delivery of lidocaine.


   **IF:** 1.194 (2016)

IV. **Mónika Bakonyi**, Attila Gácsi, Anita Kovács, Mária Budai-Szűcs, Szilvia Berkó, Erzsébet Csányi: Following-up skin penetration of lidocaine from different vehicles by Raman spectroscopic mapping.

   *Journal of Pharmaceutical and Biomedical Analysis* 154 1-6 (2018)

   **IF:** 3.255 (2016)


   *Anti-cancer Agents in Medicinal Chemistry* (accepted for publication)

   doi:10.2174/1871520617666171113120255

   **IF:** 2.598 (2016)
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PRESENTATIONS RELATED TO THE SUBJECT OF THESIS


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