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Summary of the Ph.D. thesis

Development and investigation of nanostructured lipid carriers for transdermal drug delivery

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DEVELOPMENT AND INVESTIGATION OF NANOSTRUCTURED LIPID CARRIERS FOR TRANSDERMAL DRUG DELIVERY

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1. INTRODUCTION

The topical application of drugs is a favorable administration route in the treatment of skin diseases and musculoskeletal disorders. The advantage of dermal preparations is their administration at the site where the effect is needed.

Lipid nanoparticles (LNPs) are intensively studied drug delivery systems derived from o/w emulsions, combining the advantages of polymeric nanoparticles, conventional emulsions and liposomes, while simultaneously avoiding their disadvantages. They are capable to improve the insufficient physicochemical properties of biopharmaceutical classification system (BCS) II (low water solubility, high permeability) class pharmaceutical actives, such as nonsteroidal anti-inflammatory drugs (NSAIDs), enhancing their bioavailability. The second generation of LNPs is the nanostructured lipid carriers (NLCs). The dermal use of NLC systems offers a number of advantages, such as physical stability of the applied topical formulations, enhancement of the chemical stability of the incorporated active pharmaceutical ingredients (APIs), improved dermal bioavailability, the skin targeting of the APIs, and film formation on the skin, accompanied by controlled occlusion and skin hydration *in vivo*. UV-reflecting properties (e.g. the possibility of using these carriers in sunscreens to help increase their protective effect against UV light) and the possibility of modulating API release into the skin have also been reported.

However, formulation of an NLC-based drug delivery system is a complex and long-lasting procedure, since the physicochemical properties of NLCs are altered by many factors, such as the quality and quantity of the selected lipids and surfactants or the ratio of lipids to API in the formulation. On view of their significant effects on the physicochemical properties of the nanoparticles, the selection of the proper ingredients is a crucial step in the formulation of NLCs. Optimization of the formulation via a factorial design could facilitate this process. Response surface methodology (RSM) is an appropriate tool to evaluate the correspondence between the response and independent variables and to optimize the processes or products. RSM requires less experimentation and provides estimates of the relative significance of the different variables.

2. EXPERIMENTAL AIMS

The aim of my Ph.D. work was to investigate ibuprofen-loaded nanostructured lipid carriers (IBU-NLC), optimizing the composition so as to achieve increased API penetration.

1. In the first part of my Ph.D. work, the excipients were selected and the composition was optimized in order to prepare an NLC system loaded with ibuprofen (IBU) in the highest concentration. The following aims were set:
    - to select a suitable solid lipid and a suitable liquid lipid which are compatible with IBU and incorporate it in the highest concentration;
    - to select a suitable surfactant which stabilizes the LNPs in the aqueous phase providing a stable NLC system;
    - to develop an NLC system suitable for transdermal use;
    - to optimize the composition by using a 2^3 full factorial design;
    - to evaluate the drug–excipient compatibility via:
        - differential scanning calorimetry (DSC) measurements,
        - X-ray diffraction (XRD) analysis,
        - Fourier transform infrared (FT-IR) spectroscopy measurements.
2. The second part of my Ph.D. research was to characterize the properties of the IBU-NLC. The aims were:
    - to determine the particle sizes (PSs) of the IBU-free (blank) and IBU-NLC formulations by photon correlation spectroscopy (PCS), laser diffraction (LD) and atomic force microscopy (AFM) methods;
    - to measure the zeta potential (ZP), using the electrophoretic mobility method;
    - to examine the crystalline properties of the lipid matrix and the state of the incorporated drug through XRD measurements;
    - to study the occurrence of possible interactions between the components of the prepared samples by using FT-IR and Raman spectroscopy;
    - to localize the drug in the lipid matrix via Raman mapping;
    - to study the permeability of the drug through *in vitro*, *ex vivo* and *in vivo* studies in comparison with a reference formulation.

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Ibuprofen (IBU)

NLC formulations were loaded with IBU, which was provided by PannonPharma Ltd (Pécsvárad, Hungary).

3.1.2. Solid lipids and liquid lipids

Solid and liquid lipids were examined to choose the most appropriate ones, which would dissolve IBU in the highest concentration and form a stable lipid matrix (Table 1).

Table 1: The main properties of the used solid and liquid lipids.

Lipid type	Examined lipids	Chemical name	Melting point (°C)
solid lipids	Compritol 888 ATO	glyceryl behenate/dibehenate	70–72
	Cutina CP	cetyl palmitate	55–56
	Precirol ATO 5	glycerol palmitostearate	52–55
	Witepsol E85	hard fat	42–44
liquid lipids	Miglyol 812	caprylic/capric triglyceride	< 0
	Oleic acid	oleic acid	13–14
	Walcer	special sunflower-seed oil	-18

3.1.3. Surfactants

The main properties of the surfactants used during my research work are summarized in Table 2 below:

Table 2: Main properties of the used surfactants.

Name	Chemical name	Type	HLB value
Tego Care 450	polyglyceryl-3 methylglucose distearate	ionic	12
Cremophor EL	macrogolglycerol ricinoleate	nonionic	12–14
Cremophor RH60	PEG-60 hydrogenated castor oil	nonionic	15–17
Lutrol F68	Poloxamer 188	nonionic	29
Tween 20	Polysorbate 20	nonionic	16.7
Tween 80	Polysorbate 80	nonionic	15

3.1.4. Other excipients

Carbopol 971P NF (Azelis Hungary Ltd, Budapest, Hungary) was used as gelling agent. Macrogol 400 was obtained from Hungaropharma Ltd (Budapest, Hungary). Purified water (HPLC grade) was produced with a TKA Smart2Pure system (TKA GmbH, Niederelbert, Germany), which was used to prepare all the formulations.

3.2. Preformulation studies

3.2.1. Lipid screening

Increasing concentrations of IBU were added to the solid and liquid lipids and the mixture was stirred with a Thermomixer Comfort (Eppendorf, Hamburg, Germany). The measurements were performed by a method described previously.

3.2.2. Contact angle measurements

Contact angles were measured with an Easy Drop G1 (A.Krüss Optronic GmbH, Hamburg, Germany). The contact angle of the droplet obtained from purified water (as reference) or 0.5 % (w/v) different surfactant solutions (in HPLC water) was measured on the lipid film.

3.3. Size measurements and zeta potential analysis

Particle size was analyzed by PCS using a Zetasizer Nano ZS and by LD with a Mastersizer 2000 (Malvern Instruments, Malvern, UK). Stability was predicted measuring the zeta potential (ZP) with a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK).

3.4. Atomic force microscopy (AFM)

For the size measurements and the morphological investigations of the blank and IBU-NLC samples, the tapping mode was used on a SOLVER Scanning Probe Microscope (NT-MDT Co., Zelenograd, Moscow, Russia) under ambient conditions.

3.5. Differential scanning calorimetry (DSC) measurements

DSC measurements were performed with a DSC 204 F1 Phoenix instrument (Netzsch Group, Selb, Germany). The solid lipid, the lipid mixture and the IBU-lipid mixture was examined. The measurements were performed by a method described previously. The crystallinity index (CI(%)) was calculated from the obtained DSC thermograms:

$$\mathrm{CI}(\%) = \left(\frac{\Delta H_{\text{bulk material}} \cdot \text{solid lipid ratio}}{\Delta H_{\text{solid lipid}}} \right) \cdot 100 ,$$

where ΔH is the enthalpy of the examined material.

3.6. X-ray diffraction (XRD) analysis

XRD analysis was performed with a Bruker D8 Advance diffractometer (Bruker AXS GmbH, Billerica, MA, USA) system with Cu K λI radiation (λ = 1.5406 Å). The raw materials, the physical mixtures and the NLC compositions were scanned at 40 kV and 40 mA from 3 to 40° 2θ, at a scanning speed of 0.1°/s and a step size of 0.010°.

3.7. DXR Raman spectroscopy measurements

Raman spectra were recorded with a Thermo Fisher DXR Dispersive Raman spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) attached to an Olympus MPlan 10x/0.25 BD microscope (Olympus Corp., Tokyo, Japan). IBU-NLC and blank NLC compositions were investigated by Raman mapping to localize the IBU inside the formulation.

3.8. Fourier transform infrared (FT-IR) spectroscopy

FT-IR spectra were recorded with a Bio-Rad Digilab Division FTS-65A/896 FTIR spectrometer (Bio-Rad Laboratories Inc., Hercules, CA, USA) between 4000 and 400 cm^{-1}, with 128 scan size.

3.9. Drug loading (DL%) and entrapment efficiency (EE%) measurements

The IBU-NLC sample was diluted with phosphate buffer solution (PBS) and was centrifuged. The obtained solution was filtered and injected directly into the HPLC system. DL% and EE% were evaluated by the indirect method:

$$DL\% = \frac{W_{initial\ drug} - W_{free\ drug}}{W_{lipid}} \cdot 100\%$$

$$EE\% = \frac{W_{initial\ drug} - W_{free\ drug}}{W_{initial\ drug}} \cdot 100\%,$$

where W is the weight in mg.

3.10. Drug permeability studies

3.10.1. *In vitro* release studies

The *in vitro* drug release study was carried out by using the dialysis bag method. The IBU-NLC formulation and a reference 1 % IBU suspension were examined.

3.10.2. *Ex vivo* permeation studies

The *ex vivo* permeation studies were performed with a vertical Franz diffusion cell system (Hanson Microette TM Topical & Transdermal Diffusion Cell System, Hanson Research Corporation, Chatsworth, CA, USA). 0.5% IBU-NLC gel or 0.5% IBU gel (which served as a reference) were prepared.

3.10.3. *In vivo* animal studies

The modified dorsal skin fold chamber was used to determine IBU penetration from the IBU-NLC gel and the reference IBU-gel through living animal (SKH-1 hairless mice) skin. The three measurements were performed by methods described previously.

3.11. Preparation of ibuprofen-loaded nanostructured lipid carriers

The first batch of drug free (blank NLC), and IBU-NLCs was prepared by a hot high-pressure homogenization method, using a Panda K2 NS1001L Spezial modified for NLC production (GEA Niro Soavi, Germany). The method has been described previously. Another series of NLC formulations (both blank and drug-loaded) have been prepared containing 1% IBU, using an Emulsiflex C-3 High Pressure Homogenizer (Avestin Europe GmbH, Mannheim, Germany). An IBU suspension containing 1% of IBU dispersed in purified water was prepared as a reference for *in vitro* diffusion studies of IBU-NLC. For the *ex vivo* permeation and *in vivo* animal studies IBU-NLC was gelled with a previously prepared 3% Carbopol 971P NF gel. For comparison, 0.5% IBU was dissolved in Macrogol 400 and gelled with the same polymer.

3.12. Experimental design

Eight different NLC samples (NLC 1-8) were prepared according to the 2^3 full factorial design to evaluate the effects of three factors: A (the solid lipid concentration), B (the liquid lipid concentration) and C (the surfactant concentration). The optimization parameters, (dependent factors) were the measured ZP and mean particle size ($d(0.5)$). The effects of the chosen factors were examined at two levels (+1, and -1). Besides these 8 samples, 8 other formulations, belonging in the same parameter space were prepared to check whether there was a possibility of a simpler model with which to optimize these formulations (Table 3). Statistical data analysis was performed using Statistica for Windows software version 10.

Table 3: Values of the examined independent factors (A, B and C) and dependent factors (ZP and $d(0.5)$), compositions of the NLC systems used in the factorial design (1-8) and randomly picked out from the parameter space (blank, 9-15).

Sample name	Solid lipid concentration % (w/w)	Liquid lipid concentration % (w/w)	Surfactant concentration % (w/w)	Zeta potential (mV)	Particle size (nm)
NLC 1	7 (-1)	3 (-1)	4 (-1)	-14.2	135
NLC 2	10 (+1)	3 (-1)	4 (-1)	-9.5	160
NLC 3	7 (-1)	5 (+1)	4 (-1)	-11.2	143
NLC 4	10 (+1)	5 (+1)	4 (-1)	-11.7	140
NLC 5	7 (-1)	3 (-1)	5 (+1)	-14.2	129
NLC 6	10 (+1)	3 (-1)	5 (+1)	-10.9	152
NLC 7	7 (-1)	5 (+1)	5 (+1)	-11.4	147
NLC 8	10 (+1)	5 (+1)	5 (+1)	-12.1	150
blank NLC	10	5	5	-7.54	149
NLC 9	7	3.5	4	-12.4	131
NLC 10	10	4	4	-12.3	137
NLC 11	10	4	5	-15.4	143
NLC 12	7	4.5	5	-9.64	130
NLC 13	10	4.5	4	-12.0	136
NLC 14	10	4.5	5	-12.3	141
NLC 15	7	4	4	-9.97	158

3.13. Statistical analysis

The 2-way ANOVA test (Bonferroni's multiple comparison), was used for statistical analysis (Prism 5, GraphPad Software Inc, La Jolla, CA, USA). The data are the averages of at least 5 experiments ± standard deviation (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ****$p \leq 0.0001$ versus the control).

4. RESULTS AND DISCUSSION

I. OPTIMIZATION OF THE NLC FORMULATION

4.1 Results of lipid screening

Apart from oleic acid, all of the investigated lipid melts dissolved IBU in a very high amount (50 %(w/w)). With most of the examined lipids, the IBU underwent recrystallization

(Table 4). No IBU crystals were found in Witepsol E85 and Miglyol 812, and these materials were therefore chosen as lipid matrix for NLC formulation. The solid lipid and liquid lipid mixtures were tested in ratios of 10:3, 7:3, 10:5 and 7:5. No oil droplets were observed on the filter paper. No recrystallization was observed visually after the addition of the drug.

Table 4: Results of lipid screening.

Lipid type	Examined lipids	Solubility of IBU	Recrystallization after 24 h
solid lipid	Compritol 888 ATO	soluble	yes
solid lipid	Cutina CP	soluble	yes
solid lipid	Precirol ATO 5	soluble	yes
solid lipid	Witepsol E85	soluble	no
liquid lipid	Miglyol 812	soluble	no
liquid lipid	oleic acid	insoluble	yes
liquid lipid	Walcer	soluble	yes

4.2 Results of contact angle measurements

Contact angle measurements were performed to evaluate the surfactant displaying the best wetting with the Witepsol E85 and Miglyol 812 mixture. Each of the solid lipid:liquid lipid ratios used in the factorial design (10:3, 7:3, 10:5 and 7:5) were examined. There was no significant difference between the four ratios, results for the solid lipid:liquid lipid ratio of 10:5 are shown in Fig. 1.

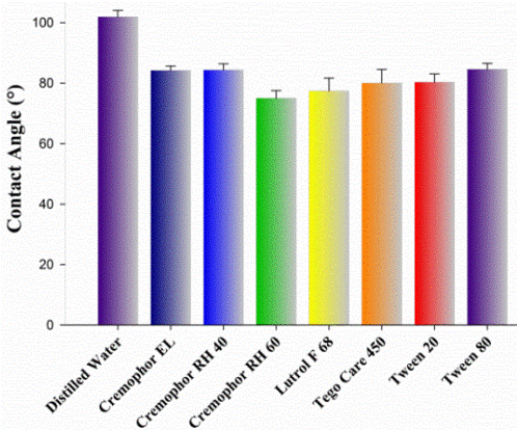

Fig. 1: Contact angles of 0.5 % (w/v) surfactant solutions applied to the 10:5 Witepsol E85:Miglyol 812 mixture.

Cremophor RH60 exhibited the best wetting properties with the lipid mixture (75.02±2.49°). Similar results could be achieved with Lutrol F68 (77.4±4.29°). NLC formulations were prepared with both emulsifiers, and Lutrol F68 was chosen for further investigations on the basis of the mean particle size ($d(0.5)$) and the zeta potential (ZP) values (Cremophor RH 60: $d(0.5)$ = 214.4 nm, ZP = -9.17 mV, Lutrol F68: $d(0.5)$ = 112.4 nm, ZP = -12.4 mV).

4.3 Results of differential scanning calorimetry (DSC) measurements

Witepsol E85 exhibited a broad endothermic event (melting range: around 42 °C). The bulk mixtures of the lipids, Witepsol E85 and Miglyol 812 in all investigated ratios gave a broader endothermic peak, with lower onset and peak values, as expected. The four lipid mixtures yielded similar DSC curves; the lipid mixture with a ratio of 10:5 is shown in Fig. 2.

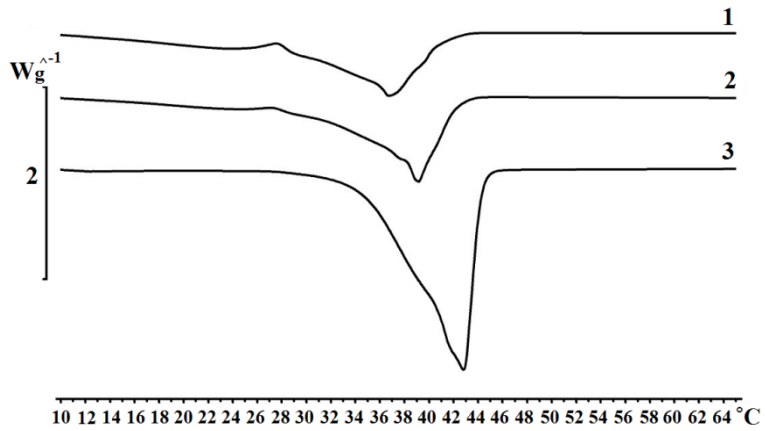

Fig. 2: DSC curves of bulk materials: Witepsol E85:Miglyol 812:IBU 10:5:5 **(1),** Witepsol E85:Miglyol 812 10:5 **(2)**, and Witepsol E85 **(3)**.

Both the addition of Miglyol 812 and the incorporation of IBU in the lipid matrix can result in an increase in the number of defects in the lipid crystal lattice, causing decreases in the CI(%) and the melting point of Witepsol E85. A lipid mixture consisting of very differently structured molecules will prevent the formation of a perfect crystal, providing spaces in which to accommodate the drug in molecular form or as amorphous clusters.

4.4 Results of X-ray diffraction (XRD) analysis

XRD measurements were performed to investigate the effects of the preparation temperature on the structures of the ingredients. The diffractogram of the melted bulk of the 10:5:5 physical mixture of Witepsol E85:Miglyol 812:IBU revealed the characteristic peaks of both the IBU and the lipid mixture. On the addition of Lutrol F68 to the mixture, a further reduction of the IBU peaks resulted (Fig. 3). It is clear from the diffractograms that both the IBU and the excipients retained their crystallinity, but the intensity of the IBU peaks decreased for both the lipid mixture and the physical mixture of the NLC components, which predicts the presence of the IBU in both dissolved and crystalline form.

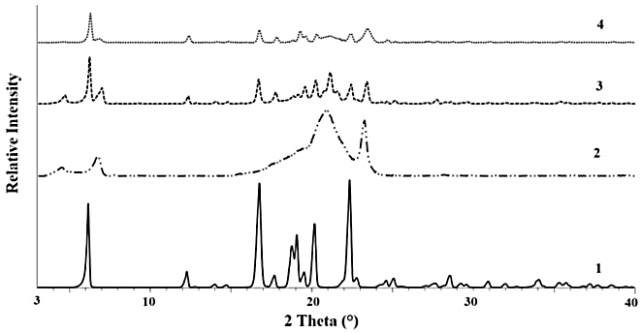

Fig. 3: X-ray diffractograms of IBU **(1)**, the 10:5 bulk mixture of Witepsol E85:Miglyol 812 **(2)**, the 10:5:5 bulk mixture of Witepsol E85:Miglyol 812:IBU **(3)**, and the 10:5:5:5 bulk mixture of Witepsol E85:Miglyol 812:IBU:Lutrol F68 **(4)**.

4.5 Results of Fourier transform infrared (FT-IR) spectroscopy

FT-IR analysis was used to verify whether any interaction occurred between the excipients and the drug because newly formed bonds can modify the diffusion of IBU from the nanoparticles (Fig. 4). A comparison of the spectra allows the conclusion that no chemical modification occurred at the temperature of NLC production. No shifts in the major valency vibrations were detected and new peaks did not appear.

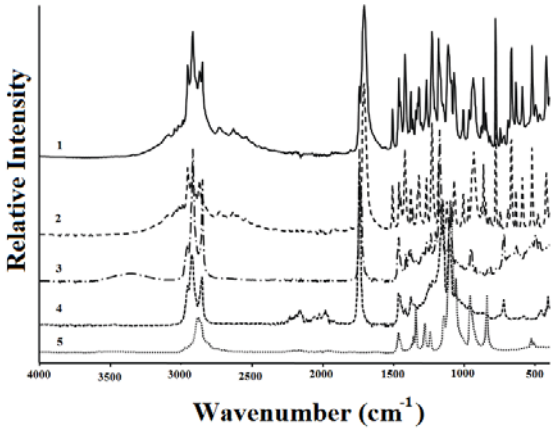

Fig. 4: FT-IR spectra of the physical mixture of the components (Witepsol E85, Miglyol 812, IBU and Lutrol F68 in a ratio of 10:5:5:5 **(1)**, IBU **(2)**, Witepsol E85 **(3)**, Miglyol 812 **(4)**, Lutrol F68 **(5)**.

4.6 Results of size measurements and zeta potential analysis

The particle size of the NLC systems varied in the interval 108.6-216.3 nm (Table 5).

Table 5: Results of PCS and LD measurements for all the NLC formulations.

Name	ZP (mV)	Z_{ave} (nm)	PDI	*d*(0.1) (nm)	*d*(0.5) (nm)	*d*(0.9) (nm)	*d*(0.95) (nm)	*d*(0.99) (nm)	Span value
blank NLC	-7.54	132.2	0.096	91	149	228	250	300	0.919
NLC 1	-14.2	216.3	0.230	97	135	249	280	330	1.126
NLC 2	-9.5	114.5	0.077	87	160	195	210	250	0.675
NLC 3	-11.2	113.1	0.067	86	143	192	210	250	0.741
NLC 4	-11.7	114.5	0.087	89	140	198	220	260	0.779
NLC 5	-14.2	108.6	0.065	86	129	192	210	250	0.822
NLC 6	-10.9	115.8	0.093	91	152	206	230	270	0.757

NLC 7	-11.4	112.7	0.089	85	147	191	210	250	0.721
NLC 8	-12.1	115.0	0.078	87	150	195	210	250	0.720
NLC 9	-12.4	112.4	0.089	87	131	195	210	250	0.824
NLC 10	-12.3	138.2	0.249	74	137	238	270	320	1.197
NLC 11	-15.4	114.7	0.108	90	143	219	240	280	0.902
NLC 12	-9.64	109.7	0.057	86	130	191	210	250	0.808
NLC 13	-12.0	116.1	0.085	90	136	200	220	260	0.809
NLC 14	-12.3	114.8	0.101	92	141	208	230	270	0.823
NLC 15	-9.97	214.4	0.237	91	158	262	300	360	1.082

4.7 Results of the experimental design

4.7.1. Effects of the dependent factors on the zeta potential (ZP) values

The three examined independent factors (concentration of solid lipid (A), liquid lipid (B) and surfactant (C)) together exerted a significant effect ($p<0.05$) on the ZP, but individually they did not do so. The coupled factors were also tested without giving a significant effect. Factor A is directly proportional to the ZP, although this effect is not significant. The ZP becomes lower if factor A is kept on level -1 (7% Witepsol E85). The same effect was observed for factor B, e.g. if 3% Miglyol 812 is applied. The ratio of factors A and B is indirectly proportional to the ZP. The response surfaces exhibit a minimum value at a solid lipid:liquid lipid ratio of 7:3, the ZP is -14.2 mV. This minimum does not change if the surfactant concentration is altered from 4% to 4.5% or 5% w/w; the behavior of the response surface remains unchanged in the examined range. Factor C is indirectly proportional to the ZP. Lower ZP values are expected if higher concentrations of the surfactant are applied; the use of 5% Lutrol F68 is therefore favorable.

4.7.2. Effects of the dependent factors on the particle size (PS)

The three independent factors together affected the PS significantly ($p<0.05$), whereas their individual effects did not reach the level of significance. No significant effect was observed when the effects of the coupled factors were tested (A and B, A and C and B and C). Factor A is directly proportional to the PS, without significant result. PS is lower if factor A is kept on level -1 (7% Witepsol E85). The same effect is observed with factor B, i.e. PS of the nanoparticles is lower when 3% Miglyol 812 is used in the formulations. The ratio of factors A and B is indirectly proportional to the PS. Factor C (surfactant concentration) did not have an impact on the PS in this range.

The results of the applied factorial design indicate that NLC 5 was the most suitable for IBU delivery, together with 7% Witepsol E85, 3% Miglyol 812 and 5% Lutrol F68.

Table 3 (chapter 3.12) gives data on NLC formulations randomly picked out from the factorial design space. By measuring the ZP, Z_{ave}, polydispersity index (PDI) and width of the PS distribution (Span value) of these NLC systems, a simpler function could be defined to

choose the optimum formulation. Factorial design requires a large number of samples, which may be decreased through use of the following equation if the response surface has a minimum value. The aim was to find a simpler correspondence to evaluate the optimum ratio of the compounds. The following equation gives the optimum as a non-dimensional number with a minimum value at the optimum compound.

$$\text{Optimum} = \min\left[\text{ZP} + Z_{ave} + \text{PDI} + \text{Span} + {}^{\text{SL}}/_{\text{LL}} + \frac{{}^{\text{SL}}/_{\text{LL}}}{\text{S}}\right],$$

where ZP = zeta potential; Z_{ave} = effective particle size; PDI = polydispersity index, Span = Span value, $\frac{\text{SL}}{\text{LL}}$ = solid lipid:liquid lipid ratio and $\frac{\text{SL/LL}}{\text{S}}$ = solid lipid:liquid lipid ratio proportional to the surfactant concentration.

As regards to the optimum equation, NLC5 (7% Witepsol E85, 3% Miglyol 812, 5% Lutrol F68 and 5% IBU) proved optimal as the composition of a stable IBU-NLC system.

II. CHARACTERIZATION OF THE IBU-NLC FORMULATION

The drug concentration of the optimized NLC5 formulation was decreased from 5% to 1% in order to decrease the side effects in diseases demanding a prolonged treatment. It was noticed that decreasing the concentration of IBU led to the increase in the stability of the formulation (the ZP values decreased from −14.2 to −18.4 mV).

4.8 Results of particle size and zeta potential measurements

Both samples were in the nanometer range, with an effective particle size (Z_{ave}) of 114 nm for the blank and 106 nm for IBU-NLC. Larger particles (>1 μm) were not present in the formulations. The surface charge was negative for both samples. The polydispersity index (PDI) was a small value for both formulations, meaning that the Z_{ave} of the nanoparticles was in a narrow range. The measured parameters measured are summarized in Table 6.

Table 6: Particle sizes, particle size distributions (PDI and Span) and zeta potentials of the blank NLC and IBU-NLC formulations.

Sample	Z_{ave} (nm)	ZP (mV)	PDI	*d*(0.1) (nm)	*d*(0.5) (nm)	*d*(0.9) (nm)	Span value
blank NLC	114 ± 2.2	-15.9 ± 0.7	0.15 ± 0.1	67 ± 0	118 ± 0	204 ± 0.6	1.16 ± 0
IBU-NLC	106 ± 1.7	-18.4 ± 1.3	0.18 ± 0.3	74 ± 0	122 ± 0	205 ± 0.6	1.07 ± 0

4.9 Results of atomic force microscopic (AFM) measurements

AFM has been widely used to acquire information on the size, shape and surface morphology of nanoparticles. The *Z* values (height, referring to the size) of the blank NLC particles were between 109 and 124 nm with an average of 113.67 ± 15.5 nm, while those of the IBU-NLC were between 95 and 118 nm, with an average of 107.47 ± 14.4 nm verifying the PCS and LD

results. In the present samples, the separated lipid particles were spherical or nearly spherical with a smooth surface. Fig. 5 presents the 2D and 3D image taken from the blank NLC.

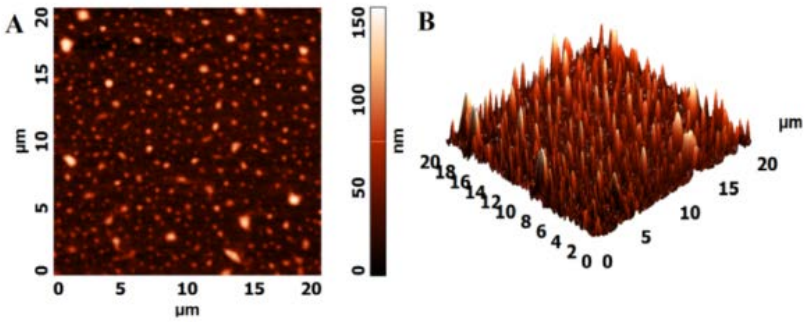

Fig. 5: 2D (A) and 3D (B) images of blank NLC.

4.10 Results of X-ray diffraction (XRD) measurements

XRD measurements were carried out to determine the possible changes in the crystallinity of the components during the hot high-pressure homogenization procedure. Diffractograms of the pure, untreated components (IBU, Witepsol E85, and Lutrol F68) are depicted in Fig. 6.

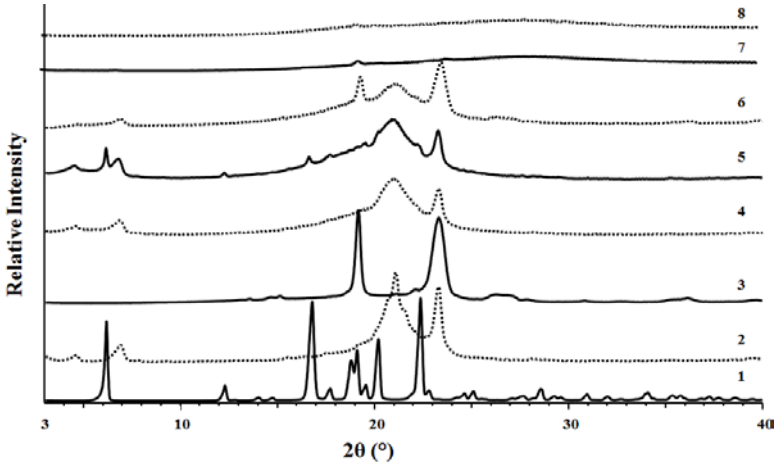

Fig. 6: XRD diffractograms of IBU **(1)**, Witepsol E85 **(2)**, Lutrol F68 **(3)**, the bulk mixture of the lipids in 7:3 ratio **(4)**, the mixture of the lipids and IBU in a ratio of 7:3:1 **(5)**, the mixture of lipids, IBU and Lutrol F68 in a ratio of 7:3:1:5 **(6)**, blank NLC **(7)**, and IBU-NLC **(8)**.

Diffractograms were also recorded of the melted lipid mixture (Witepsol E85 and Miglyol 812 in a ratio of 7:3) with or without IBU, the melted total physical mixture, the blank and IBU-NLC. The crystallinity of the solid lipid (plot 2) decreased to such an extent after the addition of the excipients (plots 4 and 6) and the drug (plot 5) that the material became amorphous in the cases of the prepared blank (plot 7) and IBU-NLC (plot 8) formulations.

4.11 Results of DXR Raman spectroscopy measurements

Raman spectroscopy was employed to confirm the physical state of the IBU and to study the possible physicochemical interactions between the components. The Raman spectra of the

excipients, the blank NLC and IBU-NLC were recorded in the wavenumber range 2000-200 cm^{-1}. The IBU spectrum exhibited characteristic peaks, which are attributed mainly to aryl ring stretching and C_{24}-Ar-C_{11} conformational stretching and wagging. Medium sharp peaks are attributed to the Ar and Ar-CH in-plane and out-of-plane bending. Spectrum of the IBU-NLC composition revealed small shifts, indicating the occurrence of weak interactions between IBU and the lipids. Spectra of the lipid components in the range 3000-200 cm^{-1} displayed characteristic peaks, which are assigned to vibrations of the fatty acid hydrocarbon chains. The incorporation of IBU did not lead to the disappearance of the sharp bands at 2881 and 2850 cm^{-1} in the Raman spectrum of IBU-NLC.

In order to confirm the homogeneity of the IBU, Raman mapping of the NLC was performed. Fig. 7 shows the distribution map of IBU in the NLC composition. The characteristic bands obtained for IBU at 1608 cm^{-1} were used to visualize the spatial distribution of IBU from Raman chemical mapping. The IBU was found homogeneously in the dried, round areas.

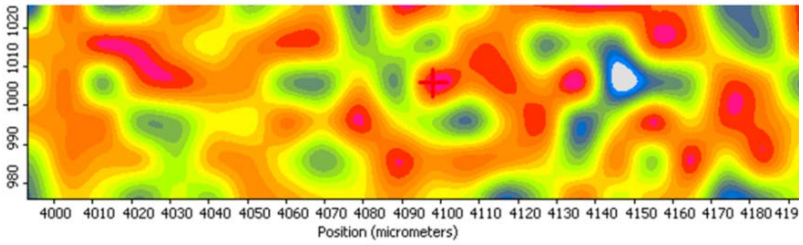

Fig. 7: Raman distribution map of IBU (yellow to red colors) in the IBU-NLC composition.

4.12 Results of Fourier transform infrared (FT-IR) spectroscopic measurements

The FT-IR spectra of the excipients, blank and IBU-NLC were recorded to obtain information about the possible interactions between IBU and the matrix of the nanoparticles. Analysis of the spectrum of IBU-NLC clearly indicates that there are no strong interactions between the drug and the excipients.

4.13 Results of drug loading (DL%) and entrapment efficiency (EE%)

From the results of the applied HPLC method, DL% was found to be 9.85 ± 4.10% and EE% 98.51±4.10% for the prepared IBU-NLC composition.

4.14 Results of drug penetration studies

The *in vitro* diffusion of IBU through the artificial membrane from IBU-NLC and the IBU suspension was calculated in terms of the mean cumulative amount diffused at each sampling time point during a period of 6 h (Fig. 8, A). The amount of IBU diffused from the IBU-NLC after 6 h was significantly higher, 2.59-fold ($p < 0.0001$) than that from the IBU suspension. The *ex vivo* permeation of IBU from the prepared IBU-NLC gel and IBU gel through excised human skin was calculated in the same way as for the *in vitro* measurements (Fig. 8, B). The

API permeation was 12.78-fold higher ($p < 0.001$) from the IBU-NLC gel than from the traditional IBU gel. The *in vivo* penetration of IBU from the IBU-NLC gel and the IBU gel was determined with a murine model, using a modified dorsal skin chamber (Fig. 8, C). After 6 h the drug penetration was significantly higher (1.87-fold, $p < 0.0001$) from the IBU-NLC gel formulation than from the IBU gel, as found in the previous *in vitro* and *ex vivo* studies.

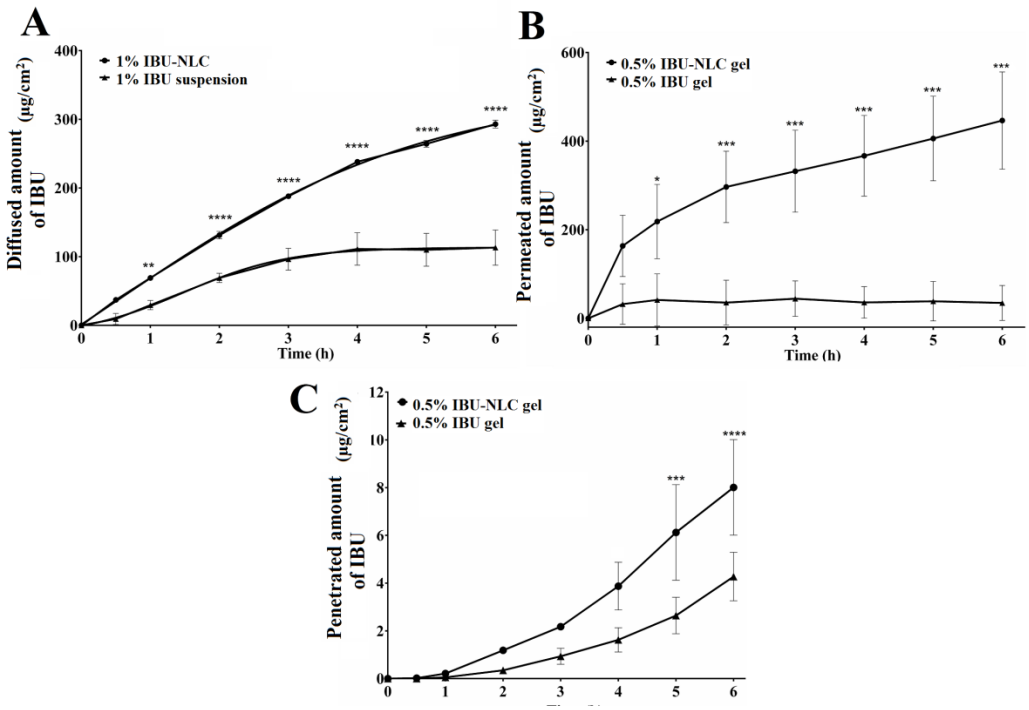

Fig. 8: **A:** *In vitro* diffusion of IBU from the IBU-NLC (●) and the IBU suspension (▲); **B**: *ex vivo* permeation of IBU from the IBU-NLC gel (●) and the IBU gel (▲) through excised human epidermis; **C**: *in vivo* penetration of IBU from IBU-NLC gel (●) and the IBU gel (▲) through living animal skin.

5. SUMMARY

The aim of my Ph.D. work was to investigate ibuprofen-loaded NLC (IBU-NLC) systems optimizing their compositions and to characterize their properties for musculoskeletal disorders needing a prolonged therapy. Summarizing my experimental work it can be concluded that:

- on the basis of lipid screening Witepsol E85 was selected as the solid lipid and Miglyol 812 as the liquid lipid which were proven to be compatible with ibuprofen and could incorporate the drug in the highest concentration, 50 % (w/w) from the examined lipids;
- based on the results of contact angle measurements, Lutrol F68 was selected as the surfactant that could provide a stable NLC system;
    - the drug–excipient compatibility was proven via: DSC, XRD and FT-IR spectroscopy measurements;

- the composition was optimized via the obtained 2^3 full factorial design and NLC 5 (7% Witepsol E85, 3% Miglyol 812 and 5% Lutrol F68) was selected for further investigations;
- an optimum equation was defined considering the effects of the excipient concentration which could serve as a useful tool selecting the optimal formulation if the surface plot has a minimum value;

In the second part, the optimized IBU-NLC formulation was characterized. It can be concluded that:

- the particle size of the blank and IBU-NLC formulations was in the nanometer range (below 205 nm) with low PDI and Span values;
- the zeta potential revealed that the particles were negatively charged and their stability could be predicted as sufficient;
- the lipid matrix was in the amorphous form and the formulations did not contain IBU in the crystalline form;
- FT-IR spectroscopy and Raman spectroscopy did not reveal any significant interactions between the components of the prepared samples predicting fast drug release from the NLC formulation;
- Raman mapping verified that IBU was homogenously distributed in the lipid matrix;
- drug permeability studies showed fast release of IBU from the nanoparticles in the beginning and were significantly higher after 6 h comparing to the prepared reference formulations *in vitro*, *ex vivo* and *in vivo*.

Novelty of this work can be summarized in the following:

- the optimum equation was first defined serving as a useful tool, to select the optimal formulation at which the surface plot has a minimum value;
- Raman mapping was used to localize an API, namely the IBU in an NLC formulation, in the lipid matrix of the IBU-NLC;
- *ex vivo* and *in vivo* drug permeation studies of the ibuprofen-loaded nanostructured lipid carrier were first compared (and found to be significantly higher after 6 h) to a reference (IBU gel) which composition mimicked the composition of those available on the market;
- the modified dorsal skin chamber was first used to prove that the IBU could permeate through the skin of SKH-1 hairless mice *in vivo*;

All of the results of the measurements performed excellently illustrated the potential of the application of the developed IBU-NLC formulation as a stable drug delivery system of IBU, providing fast release and higher skin permeability of ibuprofen which predict better bioavailability with the possibility of topical therapy for musculoskeletal disorders.

PUBLICATIONS RELATED TO THE SUBJECT OF THE THESIS

1. Szilvia Berkó, Boglárka Balázs, **Blanka Sütő**, Gábor Erős, Brigitta Gál, Anita Sztojkov-Ivanov, Codruta Soica, Piroska Szabó-Révész, Lajos Kemény, István Zupkó and Erzsébet Csányi: Monitoring of skin penetration and absorption with a new *in vivo* experimental model.
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2. **Blanka Sütő**, Sabrina Weber, Andreas Zimmer, Gabriella Farkas, András Kelemen, Mária Budai-Szűcs, Szilvia Berkó, Piroska Szabó-Révész and Erzsébet Csányi: Optimization and design of an Ibuprofen-loaded nanostructured lipid carrier with a 2^3 full factorial design.
   Chemical Engineering Research and Design 104 488–496 (2015)
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PRESENTATIONS RELATED TO THE SUBJECT OF THE THESIS

Oral presentations

1. **Blanka Sütő**, Sabrina Weber, Szilvia Berkó, Erzsébet Csányi, Piroska Révész and Andreas Zimmer: Ibuprofen loaded lipid nanoparticles (SLN and NLC) for topical delivery. *2nd Pharma DocDay*; Graz, Austria, 04. 07. 2013.

2. Berkó Szilvia, **Sütő Blanka,** Szabóné Révész Piroska and Csányi Erzsébet: Rossz vízoldékonyságú hatóanyagok formulálása szilárd lipid nanohordozók alkalmazásával. *MKE Kristályosítási és Gyógyszerformulálási Szakosztály 7. Kerekasztal Konferenciája*; Szeged, Hungary, 16-17. 05. 2014.

3. **Sütő Blanka**, Budai-Szűcs Mária, Csányi Erzsébet and Berkó Szilvia: Termoanalitika alkalmazása lipid nanorészecskék formulálásában. *MKE Termoanalitikai Szakosztályának Termikus ülése*; Szeged, Hungary, 21. 11. 2014.

4. **Blanka Sütő**, Mária Budai-Szűcs, Péter Sipos, Erzsébet Csányi, Piroska Szabó-Révész and Szilvia Berkó: Characterisation of nanostructured lipid carriers loaded with ibuprofen. *5th International Conference and Exhibition on Pharmaceutics & Novel Drug Delivery Systems*; Dubai, United Arab Emirates, 16-18. 03. 2015. *Pharmaceutica Analytica Acta* 6 (1) p. 82. (2015)

5. **Sütő Blanka**, Berkó Szilvia, Csányi Erzsébet: Nemszteroid gyulladásgátlót tartalmazó NLC rendszerek fejlesztése. *Gyógyszertechnológiai és Ipari Gyógyszerészeti Konferencia*, Siófok, Hungary, 15-17. 10. 2015.

Poster presentations

1. **Blanka Sütő**, Sabrina Weber, Gabriella Farkas, Rita Ambrus, Erzsébet Csányi, Piroska Szabóné Révész, Andreas Zimmer, and Szilvia Berkó:
   Ibuprofén tartalmú nanostrukturált lipid hordozók előállítása és vizsgálata transzdermális kezelés biztosítására.
   Congressus Pharmaceuticus Hungaricus XV., Budapest, Hungary, 10-12. 04. 2014.

2. **Blanka Sütő**, Gabriella Farkas, András Kelemen, Mária Budai-Szűcs, Szilvia Berkó, Piroska Szabó-Révész and Erzsébet Csányi:
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   Stratum Corneum VIII. Cardiff, United Kingdom, 17-19. 09. 2014. *International Journal of Cosmetic Science* 37 (1) pp. 159-160. (2015)

3. **Blanka Sütő**, Nikolett Ungvári, Erzsébet. Csányi, Piroska Szabó-Révész and Szilvia Berkó:
   Factorial design aided development of salicylic acid loaded nanostrucured lipid carriers.
   1st European Conference on Pharmaceutics: Drug delivery, Reims, France, 13-14. 04. 2015.