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Summary of the Ph.D. thesis

INCREASING THE OPHTHALMIC BIOAVAILABILITY USING INNOVATIVE DRUG DELIVERY SYSTEMS

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Increasing the ophthalmic bioavailability using innovative drug delivery systems

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1. INTRODUCTION

There is an increasing global need to develop new non-invasive ophthalmic treatments as there are many poorly understood and sometimes incurable vision-threatening diseases and conditions. The most common ocular diseases are glaucoma, infections, dry eye syndrome, allergies, corneal neovascularization, corneal erosion, inflammation, and macular degeneration. Most of the current medical treatments are mainly limited to conventional medicines, and in many cases, the treatment only affects the symptoms of the disease, not the underlying cause. Currently, advanced ophthalmic therapies do not just have to mean to treat various eye diseases but also have to be able to improve patient compliance or restore the healthy state of the eye. Topical ophthalmic treatments are considered the safest and the least invasive therapies, and they are easy to handle for patients. However, due to their low bioavailability, there is a great need for their development. There are several difficulties to the successful and effective delivery of the active ingredient to the eye. These include, for example, limiting factors due to the anatomical complexity and biopharmacy of the eye, and challenges related to patient compliance, e.g. inconvenience of frequent usage. Consequently, these aspects, in any case, should be taken into account when developing a new ophthalmic drug delivery system.

In recent technological developments, there are three main strategies to achieve the above-mentioned goals: in one case, the residence time of the preparation on the surface of the eye is increased; in another case, the carrier contains the active ingredient in such a form that it is already suitable for permeation (increased solubility); in the third case, the penetration of the drug is improved. During the course of my Ph.D. research, the development of ocular delivery systems was in line with the above-mentioned strategies.

2. EXPERIMENTAL AIMS

In my PhD work, the aim was to increase the bioavailability of topically applied ophthalmic preparations, which I did along with two main directions.

In the first part of my PhD work, I aimed to combine the good adhesion properties of the mucoadhesive polymers (thiolated poly(aspartic acid)) with increased solubility of corticosteroids using cyclodextrins (CDs), thereby increasing the bioavailability of the active ingredient. The possible advantages to using a modified polymer can be the *in situ* gellable and strong mucoadhesive behaviour derived from the basic polymer properties and the increased solubility of prednisolone (PR) thanks to the grafted CD. In this case, the covalently bonded drug-CD complex may not diffuse and wash out with the lacrimal drainage from the adherent layer of the ocular gel, therefore the residence time of the formulation is increased, and improved bioavailability of the lipophilic ocular drug, PR can be provided.

The second part of my PhD work can be separated into two main section. In the first section, the aim was to improve the bioavailability of dexamethasone (DXM) by dissolving in lipids and incorporate into nanostructured lipid carriers (NLCs). In the second section, the aim was to increase the ocular residence time of our formulations using a mucoadhesive polymer. A mucoadhesive gel layer can form around the nanoparticles because the amphiphilic characteristics of the polymer can result in its enrichment at the interface of the nanocarrier. This way the gel layer around the nanocarrier can ensure the adhesion of the nanoparticles to the mucosal surface.

3. MATERIALS AND METHODS

3.1 Materials

3.1.1. Materials of in situ gelling systems

L-aspartic acid, cysteamine, dibutylamine, dithiothreitol, *N*,*N*-dimethylformamide, sodium bromate, 6-monodeoxy-6-monoamino-beta-cyclodextrin hydrochloride (MABCD) and mucin (porcine gastric mucin type II) were used.

The synthesized thiolated poly(aspartic acid) (PASP-CEA) contains thiol side groups and in the presence of the oxidizing agent, PASP-CEA is reversibly cross-linked via disulphide bonds. Cyclodextrin-modified thiolated polyaspartic acid (PASP-CEA-CD) is able to form inclusion complexes with certain drugs, such as steroidal anti-inflammatory drugs, due to cyclodextrin. The thiolated poly(aspartic acid) polymers were synthesized by Budapest University of Technology and Economics, Soft Matters Group.

The applied active agents were prednisolone (PR).

3.1.2. Materials of NLC systems

Compritol 888ATO (glycerol dibehenate), Apifil (PEG-8 beeswax) Labrasol (Caprylocaproyl Polyoxyl-8 glycerides), Miglyol 812N (capric triglyceride), Kolliphor EL (Polyoxyl 35 hydrogenated castor oil) and Cremophor RH60 (PEG-60 hydrogenated castor oil), Tween 20 (Polysorbate 20), hypromellose (Methocel F4M, HPMC) were used. The API was dexamethasone.

3.2. Methods of investigation of thiolated polymers

3.2.1. Preformulation study

As preformulation study the osmolality, refractive index and pH were measured in aqueous solutions of PASP-CEA and PASP-CEA-CD at 10% w/v. The osmolality of the polymer solutions was measured using an automatic osmometer. The pH of the polymer solutions was measured with a pH-meter.

3.2.2. X-ray diffraction study of cyclodextrin-prednisolone complexes

The inclusion complexes were characterized by an X-ray powder diffractometer (XRD). The examined samples were PR, PASP-CEA-CD powder, and the lyophilized supernatant of the solution of MABCD-PR (2.5% w/v MABCD, 0.4 % w/v PR) and PASP-CEA-CD–PR (10.2% w/v PASP-CEA-CD, 0.15% w/v PR). After the sample preparation, the formulations were freeze-dried.

3.2.3. Rheological measurements

Gelation was followed by oscillatory rheological tests and gels were characterized also by rheometry. The oxidizing circumstance in the eye was simulated with sodium bromate solution. In the gelation measurements, the polymer solutions (10% w/w) were mixed with 1 M oxidant (20 % w/w) on the plate of the rheometer and the measurement was started immediately.

To study mucoadhesion, the polymer solutions were mixed with the mucin dispersion (5% w/w) in PBS before the addition of the oxidant. The rheological synergism was calculated.

3.2.4. Drug release study

The drug diffusion profile of PR was determined with a vertical Franz diffusion cell system. The donor phase was a formulation containing 10 w/w % polymer (PASP-CEA or PASP-CEA-CD), 0.1 w/w % PR and 20 w/w % oxidant solution. The gelation occurred *in situ* after the installation. The diffusion membrane was a Porafilm membrane (pore size of 0.45 μm). The acceptor phase was PBS (pH = 7.4) and it was thermostated at 35 °C. The PR released was measured at 247 nm by a UV-VIS spectrophotometer.

3.3. Methods of investigation of NLC systems

3.3.1. Preparation of NLC systems

For the preparation of the NLC samples (Table 1.), an ultrasonication method was used. The lipids (Compritol 888 ATO and Miglyol 812N at 7:3 ratio) and surfactant (Cremophor RH40) were melted with a heating magnetic stirrer at 85 °C. After that, DXM and the polymer (hypromellose: Methocel F4M) were added to the melted mixture and stirred at the temperature of 85 °C. Pure water was heated at 85 °C and the two phases were mixed and ultrasonicated and cooled in an ice bath.

Table 1. *The composition of NLC systems.*

		Lipid concentration (w/w%)	**DXM concentration (w/w%)**	**Surfactant concentration (w/w%)**	**Polymer concentration (w/w%)**
Basic NLC formulation (Factorial design 1)	NLC1	10	0.05	2.5	-
	NLC2	15	0.05	2.5	-
	NLC3	10	0.10	2.5	-
	NLC4	15	0.10	2.5	-
	NLC5	10	0.05	5.0	-
	NLC6	15	0.05	5.0	-
	NLC7	10	0.10	5.0	-
	NLC8	15	0.10	5.0	-
Mucoadhesive NLC formulation (Factorial design 2)	NLC9	10	0.05	2.5	0.05
	NLC10	10	0.05	2.5	0.10
	NLC11	10	0.05	5.0	0.05
	NLC12	10	0.05	5.0	0.10
	NLC13	10	0.10	2.5	0.05
	NLC14	10	0.10	2.5	0.10
	NLC15	10	0.10	5.0	0.05
	NLC16	10	0.10	5.0	0.10

3.3.2. X-ray diffraction of lipid compositions

The solid-state of DXM in the lyophilized lipid composition and the freeze-dried NLC formulations were analyzed with XRPD.

3.3.3. Investigation of lipid crystallinity with DSC measurements

The crystallinity index of lipid mixtures and DXM containing lipid mixtures were examined with DSC.

3.3.4. Particle size and zeta potential of NLCs

The hydrodynamic diameter (Z_{ave}), zeta potential (ZP), and polydispersity index (PI) of NLCs were investigated by the Zetasizer Nano ZS instrument with at 25 °C.

The NLCs (Table 1.) were also analyzed by particle size and particle size distribution measurements using laser diffraction. The d(0.1), d(0.5) and d(0.9) were assessed.

3.3.5. Entrapment efficacy of NLCs

The entrapment efficacy (EE%) of NLCs was determined with an indirect method. The clear aqueous phase of the NLCs was separated by centrifugation. The DXM content of the filtered solution was examined by HPLC, which was equipped with a C18 reverse-phase column with dimensions of 1.7 µm, 100 Å, 100 * 2.1 mm. The mobile phase was water: acetonitrile 75:25 in isocratic elution, the detection was made at 240 nm. The injection volume was 5 µl and the flow rate was 0.5 ml/min. The following equation was used to calculate EE%:

$$EE\ \% = \frac{W_{initial\ drug} - W_{free\ drug}}{W_{initial\ drug}} \times 100 \quad (1)$$

3.3.6. In vitro drug release study

Considering the results of the factorial experimental design, four NLC compositions were selected for diffusion study. To investigate *in vitro* drug release, the dialysis bag method was used. The applied sample amount was 200 µl. The acceptor phase was 20 ml of phosphate-buffered saline (PBS). As reference preparation, a DXM suspension was applied. The diffused amounts of DXM were analyzed by HPLC.

3.3.7. Human HCE-T corneal epithelial cell line

Human corneal epithelial cells were immortalised by transfection with a recombinant SV40-adenovirus vector.

3.3.8. Cell viability measurements

Real-time cell electronic sensing was used to follow cell damage and/or protection in living barrier-forming cells. The investigated samples were Cremophor RH60, NLC3, NLC7, NLC9, NLC10, NLC11, NLC12 formulations and HPMC solution.

3.3.9. Immunohistochemistry

Morphological changes in HCE-T cells were investigated by immunostaining for junctional proteins, zonula occludens protein-1, occludin, β-catenin and E-cadherin. Cells were grown on culture inserts used for permeability experiments.

3.3.10. Experimental design

To characterize the polymer containing NLC compositions, a 2^3 full factorial design was applied (eq. 2). The model describes the principal effects and interaction among the identified variables.

$$y = a_0 + a_1x_1 + a_2x_2 + a_3x_3 + a_{12}x_1x_2 + a_{23}x_2x_3 + a_{13}x_1x_3 \quad (2)$$

where a_0 is the intercept, $a_{1,2,3}$ were the regression coefficients values. x_1, x_2, and x_3 correspond to factors A, B and C, respectively.

In the first factorial experimental design (basic formulation), the independent factors were A (lipid concentration), B (DXM concentration) and C (surfactant concentration). In the second design (mucoadhesive formulation), the independent factors were A (polymer concentration), B (DXM concentration) and C (surfactant concentration). The chosen factors were examined at two levels (+1 and -1).

3.3.11. Mucoadhesion study

The mucoadhesion of the formulations with polymer and polymer-free were investigated with a Texture Analyzer. The work of adhesion (A, mN mm) was used to characterize the mucoadhesive behaviour.

3.3.12. Penetration study on corneal-PAMPA model

For the *in vitro* transcorneal permeability measurement, a corneal-PAMPA method was applied. DXM and its formulations were used as a donor solution. The donor plate was fit into the acceptor plate containing 300 μL of PBS solution (pH 7.4), and 150–150 μL of the PBS solutions were put on the membrane of the donor plate. The plates were incubated for 4 h at 35 °C. The effective permeability and membrane retention of drugs were calculated using the following equation (Eq. 3):

$$P_e = -\frac{2.303 \cdot V_A}{A(t-\tau_{SS})} \cdot \log\left[1 - \frac{c_A(t)}{S}\right] \quad (3)$$

where P_e is the effective permeability coefficient (cm s^{-1}), A is the filter area (0.24 cm^2), VA is the volume of the acceptor phase (0.3 cm^3), t is the incubation time (s), τ_{SS} is the time to reach steady-state (s), $C_A(t)$ is the concentration of the compound in the acceptor phase at time point t (mol cm^{-3}), S is the free drug content of DXM in the donor phase.

3.3.13. Permeability study on cell culture model

Transepithelial electrical resistance (TEER) was measured to check the barrier integrity. TEER of cell-free inserts was subtracted from the measured data. Cells were treated when the cell layer had reached steady TEER values.

The apparent permeability coefficients (P_{app}) were calculated. For permeability measurements, NLC5, NLC6, NLC7, NLC8 formulations were applied.

3.3.14. Penetration study on porcine cornea

The *ex vivo* penetration test was examined with Raman microscopy. The pig cornea was instilled with 250 μL of NLC sample (NLC14 and the polymer-free version of NLC14) every 30 min and the formulation was removed just before the next instillation. The duration of

treatment was six hours. The treated cornea was frozen and divided into cross-section. The untreated porcine cornea was used as a reference. During the evaluation, profiling of the Raman map was performed using the entire NLC spectrum.

3.3.15. Statistical analysis

The release test results were analyzed statistically with two-way ANOVA analysis with Bonferroni post-tests. For the MTT and cell penetration assays, the values were compared using ANOVA followed by Dunett's test or 2-way ANOVA followed by the Bonferroni test. The values are expressed as means ± standard deviation (SD). The factorial experimental designs were evaluated with Statistica for Windows, version 10.

4. RESULTS

4.1. Investigation of thiolated polymers

4.1.1. Preformulation study

4.1.1.1. Formation of inclusion complexes

The inclusion of PR within MABCD and PASP-CEA-CD was investigated by XRPD. Diffractograms of PR, MABCD, PASP-CEA-CD, MABCD-PR and PASP-CEA-CD-PR were recorded. The diffractogram of PR showed a crystalline structure, indicated by the sharp peaks in the graph. In the case of polymer (PASP-CEA-CD) and cyclodextrin (MABCD), an amorphous pattern can be observed, there is no high intensive characteristic peak in the diffractogram. In the case of inclusion complexes, no characteristic peak of PR can be seen in the pattern, the amorphous structure of PASP-CEA-CD and/or MABCD dominates. The absence of the crystalline peaks of PR can prove the formation of inclusion complexes.

4.1.1.2. Measurement of osmolality, pH and refractive index

The physiological characteristics of the PAS-CEA and PASP-CEA-CD solution were measured and compared with each other and the tear fluid. The osmolality of the tear film in a normal eye is 300 to 310 $mOsmL^{-1}$. In our case, the osmolality of neither polymer solution was measurable at the applied concentration with this methodology, which indicates the possibility and the necessity of the addition of excipients, such as an isotonizing agent. In some cases, the hypoosmolality of the ophthalmic solution is required, especially in artificial tears in the treatment of dry eye disease.

The refractive indexes of the polymer solution (PASP-CEA: 1.3494; PASP-CEA-CD: 1.3478) were similar to the tear film (1.3370), which suggests the formulation does not disturb the vision of the patient.

The pH range of 6 to 9 is tolerable for the eye can be ensured using pH = 7.4 PBS solution.

4.1.2. Gelation and mucoadhesivity

The gelation process, the gel structure and the mucoadhesion of the modified polymer were characterized using rheology. Gelation time is a critical factor in the case of an *in situ* gelling ophthalmic formulation. The PASP-CEA solution displayed a fast solution-to-gel transition in the presence of an oxidant (Fig 1. a).

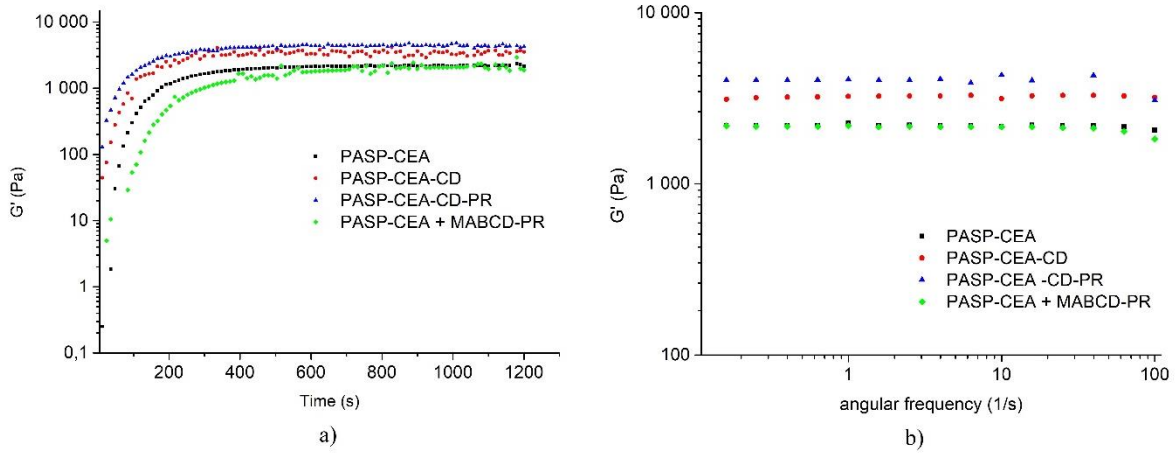

Figure 1*. Gelation (a) and frequency sweep test (b) of the different polymer solutions.*

Fig. 1. (a) demonstrates that the immobilization of MABCD on the polymers did not hinder the gelation process and the gel structure has remained.

Mucoadhesion was characterized using the synergism parameter calculated from the G' values at 1.0 1/s angular frequency: In our calculation, the synergism parameters of the PASP-CEA and PASP-CEA-CD were 1370 and 1390 Pa, respectively. The similar data mean similar mucoadhesivity, thus as a conclusion, the modification of the polymer with MABCD did not change the mucoadhesive characteristics of the PASP-CEA polymers.

4.1.3. Drug release study

The complexation of PR with CDs improves PR solubility in an aqueous medium. The complexes diffuse in the formulation and can carry the PR molecules through the aqueous mucin layer. However, it can be washed out with the drainage of the eye.

In my work, the release profile from the grafted and the free CD systems were compared *in vitro* (Fig. 2). The release profile of PASP-CEA gel formulation containing free MABCD-PR was very similar to that of PR suspension (no significant differences, $p > 0.05$). When MABCD was covalently attached to PASP-CEA, the diffusion of the complex was blocked, only free PR was able to diffuse through the membrane, thus the release rate was slower and depended on the PR dissociation from the CD molecule. To accelerate the amount of released

drug, 50% of MABCD was applied in free form. In this case, an intermediate drug release profile between the grafted and free MABCD can be observed, which indicates that with the combination of the free and grafted MABCD, the drug release profile can be modified within the two limiting profiles.

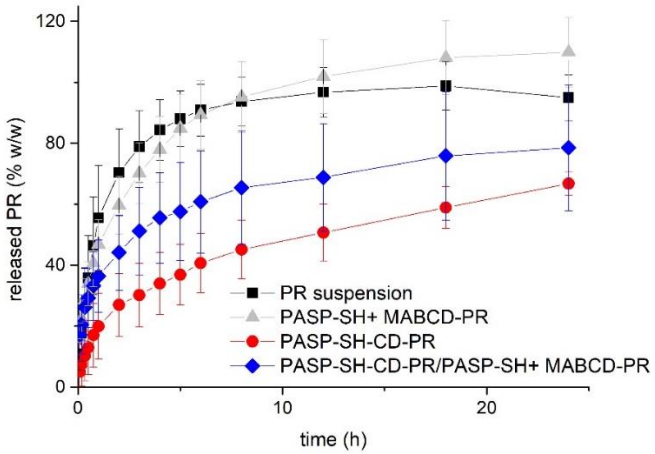

Figure 2*. Drug release from the formulations containing PR.*

4.2. Investigation of dexamethasone loaded NLC formulations

4.2.1. Preformulation of NLC systems

4.2.1.1. Lipid screening

NLCs contain solid lipid, liquid lipid and surfactant in an aqueous medium. The lipid screening focused on 3 potential critical factors. The first was to find lipid compositions which can solve the required dose of DXM. The second critical factor can be the crystallinity of the lipids because it may affect the drug loading capacity of NLCs. The third critical factor is the ability of the lipid-surfactant blends to form an NLC formulation.

4.2.1.2. Visual observations of DXM solubility

In this part of the solubility study, the visual observation of different combinations of lipid melts: solid lipids, liquid lipids alone and their mixtures with and without surfactant were applied. There was no difference between the two solid lipids (Compritol 888 ATO and Apifil). The results of the comparison of Miglyol and Labrasol showed that Miglyol was much more effective at dissolving the API. In the case of solid lipid (Compritol 888 ATO), oils (Miglyol, Labrasol) and surfactant (Cremophor RH60, Kolliphor EL) containing mixtures, DXM was dissolved in almost all cases, where the mixtures were made with Apifil, Miglyol, Cremophor RH60, Kolliphor EL.

4.2.1.3. XRPD analysis of the lipid-API mixtures

The fact that the characteristic peaks of crystalline DXM are not detectable can prove that the API is in amorf or molecularly dispersed in all the lipid matrices.

4.2.1.4. Investigation of lipid crystallinity with DSC measurements

The two emulsifiers (Cremophor RH60 and Kolliphor EL) reduced the crystallinity index, but there was no remarkable difference between them. The higher liquid lipid concentration resulted in the most relevant crystallinity index (CI%) depression. Thanks to the favourable CI% depression effect of the 7:3 solid lipid-oil ratio (with minimal melting point depression), this ratio was applied for further optimization.

4.2.1.5. Selection of the potential NLC components

To clarify the ability of the chosen lipid mixtures to form a nanosystem, drug-free NLCs were made. To characterize the NLC systems, the following factors were measured using the laser diffraction method: d(0.1), d(0.5),d(0.9) and Span values.

Based on our results, Compritol 888 ATO, Miglyol 812N, Cremophor RH60 were chosen for further measurements and formulation optimization.

4.2.2. Optimization of DXM-loaded NLCs with factorial experimental design

4.2.2.1. Basic NLC formulation

To evaluate the effect of the component concentration and find the optimal ratio, a 2^3 full factorial experimental design was used. Three formulation parameters of DXM-NLC were chosen as independent factors (the concentration of the emulsifier, the drug and the total lipid). As for the optimization parameters (dependent factors), the particle size (Z_{ave}), zeta potential (ZP), polydispersity index (PDI) and entrapment efficacy (EE%) of the NLC systems were chosen.

Based on the results, the surfactant concentration has the most remarkable effect on the stability parameter (Z_{ave} and ZP) of NLCs, an increased amount can be favourable, but we must bear in mind that its higher concentration can result in lower drug loading capacity and potential irritation and toxicity. Concerning the stability of the system, 10% lipid concentration was applied in the further compositions.

4.2.2.2. Mucoadhesive NLC formulation

To formulate a DXM containing mucoadhesive NLC system, HPMC was applied as a mucoadhesive agent, which supposedly forms a gel layer around the nanoparticles. A new 2^3 full factorial experimental design was used to optimize the mucoadhesive formulations. The chosen factors were polymer concentration (A), surfactant concentration (B), and DXM concentration (C). The optimization parameters (dependent factors) for the characterization of nanoparticles, the particle size (Z_{ave}), zeta potential (ZP), polydispersity index (PDI), d(0.1),

d(0.5), d(0.9), Span value, entrapment efficacy (EE%) and mucoadhesivity of the NLC systems were chosen.

Equations (9-14) show the effect of the chosen factors on the optimization parameters and the combined factors on optimization parameters (Zave, PDI, ZP, EE%, d(0.5) and Span value). The bold values in the equations indicate a significant effect.

Z_{ave}= 153.22 **– 4.93A** – **42.65B** – **1.74C** – **5.50AB** + **5.71AC** – 0.35BC, (4)

PDI = 0.30 – 0.03A – 0.04B – 0.02C – 0.01AB + 0.04AC + 0.00BC, (5)

ZP = -7.65+0.46A+2.21B-0.44C+0.24AB+0.83AC-1.49BC, (6)

EE% = 88.63 - 0.51A+3.6B+0.35C-0.69AB+0.51AC-1.18BC, (7)

d(0.5) = 0.12 – 0.001A -0.003B + 0.002C + 0.000AB +0.000AC+0.002BC, (8)

Span value=1.24-0.05A-0.1B+0.11C-0.09AB+0.01AC+0.1BC. (9)

Based on the results of the factorial design, surfactant concentration, polymer concentration and DXM concentration have a significant effect on Z_{ave} (eq. 4). The combined effects of the polymer and surfactant concentration (AC), and the polymer and DXM concentration (AB) also have a significant effect on Z_{ave}.

4.2.3. Mucoadhesion study

To improve the mucoadhesivity of the formulations, a mucoadhesive polymer, HPMC was added to the NLC systems. The mucoadhesion of the NLC compositions was compared with the same compositions without HPMC and then statistical analysis (T-test) was applied to evaluate the significance. It was found that there is a significant difference between samples with and without the mucoadhesive polymer in the case of most compositions. The samples containing a higher surfactant amount (5%) displayed higher adhesive work values than the compositions with a lower surfactant amount.

4.2.4. In vitro drug release study

4.2.4.1. Basic NLC formulations

To compare our optimized NLCs with the conventional suspension form, *in vitro* drug release studies were performed. Based on the results of our factorial experimental design, the four most relevant (NLC1, 5 - 0.05 % DXM and NLC3,7 - 0.10 % DXM) compositions were chosen to compare with DXM suspension.

Based on the results the NLC7 composition displayed a significantly higher amount of the released drug than the suspension form at each time point.

4.2.4.2. Mucoadhesive NLC formulations

The *in vitro* drug release from HPMC containing NLC systems were also investigated. The compositions were the same as in the factorial experimental design (NLC9-16). Considering the results of the factorial design, the surfactant concentration (B) has a significant negative effect on diffusion (eq. 10), whereas the DXM concentration (C) has a significant positive effect on diffusion.

$$C_{DXM\ (6h)} = 73.50 + 1.64A - \mathbf{14.40B + 25.07C} - 1.24AB + 2.65AC - 3.11BC, \quad (10)$$

The highest diffused amount of DXM was detectable in the case of NLC13 and 14 (which contain a lower amount of surfactant, and the lowest entrapment efficacy). This finding could show us that if less API is entrapped in the nanocarriers (more free DXM), a higher amount will diffuse through the synthetic membrane, and on the other hand, it can mean that the high emulsifier concentration (and/or high entrapment efficiency) in the system can have a restraining effect on DXM diffusion.

4.2.5. Cell viability assay

4.2.5.1. Basic NLC formulations

To analyse the possible toxicity of the formulated NLCs, a human cornea cell viability study was applied using MTT assay. The results presented formulated NLCs were non-toxic for different cultured epithelial cells.

4.2.5.2. Mucoadhesive NLC formulations

The full NLC formulations (NLC9-12) and the polymer alone were evaluated using impedance measurement, which did not show significant cell damage after treatment with different DXM containing NLC formulations.

4.2.6. Immunohistochemistry

The cell viability test was supplemented with immunohistochemistry to clarify the effect of the formulations on the barrier function of human corneal epithelial cell layers. No major morphological changes were observed in the treated groups. All junctional proteins were localized at the intercellular connections forming continuous pericellular belts in every group.

4.2.7. Penetration studies

Permeability is the ability of drug molecules to penetrate through the biological membranes. In our study 3 different methods were applied and compared with each other, such as *in vitro* high throughput corneal-PAMPA; permeability study on cell culture; and *ex vivo* penetration study on porcine cornea followed by Raman correlation mapping.

4.2.7.1. Application of corneal-PAMPA model

The comparison of the flux values of the NLC systems with the suspension indicated remarkable penetration when DXM was incorporated in nanocarriers. Surprisingly, NLC11 and 12 showed the highest penetration and a highly significant difference from the DXM suspension form. However, the NLC11 and 12 formulations showed a lower drug diffusion during the *in vitro* drug diffusion study. This contradiction may be explained by the interaction between the components of the NLCs and the cornea-PAMPA membrane.

4.2.7.2. Permeability study on cell culture model

The highest amount diffused in the case of the NLC13 and NLC14 formulations (Fig. 3.). After 60 minutes, the diffused amount of DXM was significantly higher for NLC14 than for the suspension form. When there was a higher free concentration in the donor phase, the amount of diffused DXM was also higher. This tendency is like that in the case of in vitro drug diffusion study. This may suggest that if the entrapment efficiency is higher, DXM can diffuse in a lower amount from the NLC systems.

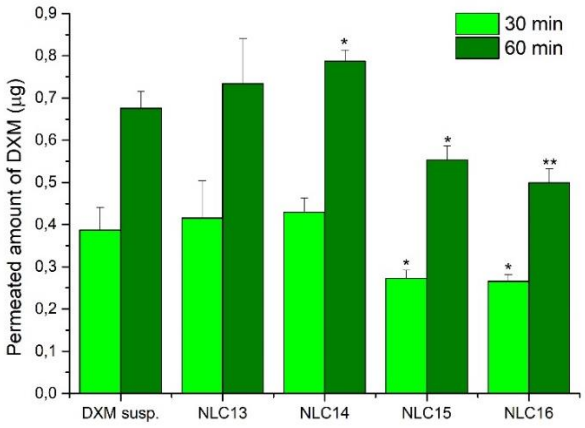

Figure 3*. The penetrated amount of DXM through HCE-T cells after 30 min and 60 min. Values are presented as means ± SD. Statistical analysis: ANOVA followed by Dunnett's test.*

Considering the permeability (P_{app}) values after treatment, all NLC formulations showed higher values compared to the suspension, which indicates a better penetration of the API using nano lipid compositions.

4.2.7.3. Correlation between the different penetration models

To evaluate the penetration studies, the correlation of the penetrated amount of drug through different membranes was analysed. Correlated results may confirm the relevance and applicability of the models and can help to choose the most suitable one to predict the bioavailability of the API in NLCs.

The results of the *in vitro* diffusion study and penetrated amount of DXM though Corneal-PAMPA does not correlate with each other (the correlation coefficient was 0.06522),

while the correlation between the results of penetrated DXM though HCE-T cells and the penetrated amount though Corneal-PAMPA was moderate (the correlation coefficient was 0.52248). The possible explanation can be that the component of the NLC can modify the PAMPA membrane, which can alter the permeability in this model.

The correlation between the *in vitro* diffusion study and the HCE-T cell line permeability study was very high (0.96595), which indicates a strong correlation. Therefore, the main rate-limiting factor for the penetration of DXM in NLC formulations is the drug release from the nanocarriers.

4.2.7.4. Ex vivo Raman correlation mapping

Based on the drug diffusion study and the cell line penetration test, NLC14 was selected for the semi-quantitative Raman mapping examination. The porcine corneas were treated with the formulations with (NLC14), and without polymer. In the case of the polymer-free NLC, we can observe a significant amount of components (surfactant, lipids) of NLCs in the stroma layer (300 μm penetration depth) of the cornea (Fig. 4.).

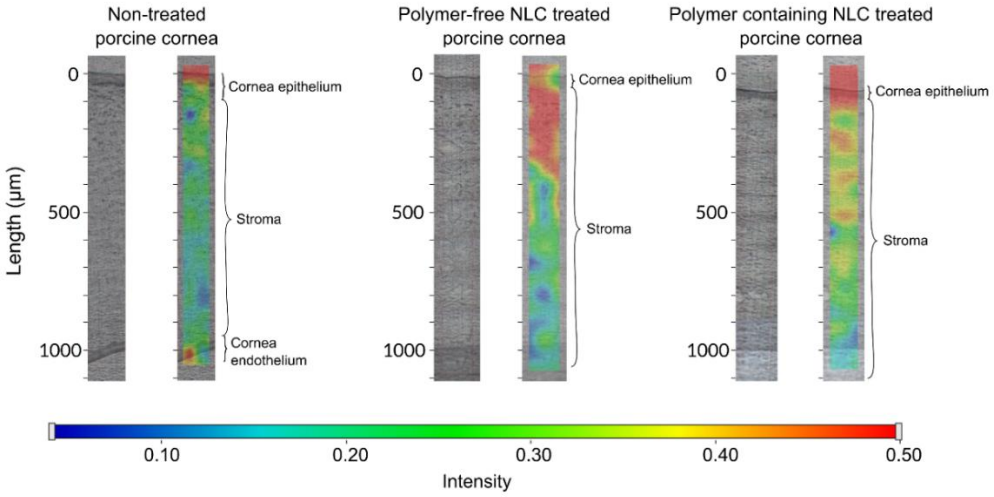

Figure 4*. The Raman correlation map of NLC14 and the polymer-free version of the composition.*

As for the polymer containing NLC (NLC14), a very remarkable Raman intensity can be seen on the surface of the cornea and in the upper part of the stroma, but the latter is less expressed than in the case of the NLC without polymer. Thanks to its adhesive properties, NLC14 might form a very significant depot on the corneal surface, and it presented fewer transitions of components through the epithelial layer into the hydrophilic stroma.

5. SUMMARY

My PhD work aimed to formulate innovative drug delivery systems for ophthalmic use. The novelty of this work can be summarized as follows:

- Formulation of in situ gelling delivery with the cyclodextrin modified thiolated polymer, which can make it possible to incorporate prednisolone as the active ingredient in a hydrophilic mucoadhesive ophthalmic composition.
- The binding of cyclodextrin to the polymer increased the duration of the effect and became programmable by changing the ratios of the free and bound forms of the cyclodextrin.
- The optimal composition of DXM loaded mucoadhesive NLC systems, which increased the possible residence time on the eye surface, was first described.
- The biocompatibility of chosen components to formulate NLC systems were verified with the immunohistochemistry method and cell viability assay.
- A strong correlation was found between *in vitro* drug release study and permeability on HCE-T cells.
- *Ex vivo* Raman mapping was applied in order to follow the penetration trough cornea, and it was proved that the components of basic NLC formulations could penetrate the stroma layer, while in the case of polymer containing NLC formulations, the system could create a depot on the eye surface.

To sum up, it can be concluded that the thiolated mucoadhesive hydrogels and NLCs described in the thesis have the possibility to increase the bioavailability of the ophthalmic steroidal anti-inflammatory drug.

PUBLICATIONS RELATED TO THE SUBJECT OF THE THESIS

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II. **Eszter, L. Kiss**; Szilvia, Berkó; Attila, Gácsi; Anita, Kovács; Gábor, Katona; Judit, Soós; Erzsébet, Csányi; Ilona, Gróf; András, Harazin; Mária A., Deli, and Mária, Budai-Szűcs; Design and Optimization of Nanostructured Lipid Carrier Containing Dexamethasone for Ophthalmic Use. Pharmaceutics 11, 679 (2019).

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III. **Eszter, L. Kiss**; Szilvia, Berkó; Attila, Gácsi; Anita, Kovács; Gábor, Katona; Judit, Soós; Erzsébet, Csányi; Ilona, Gróf; András, Harazin; Mária A., Deli, György T. Balogh and Mária, Budai-Szűcs; Development and Characterization of Potential Ocular Mucoadhesive Nano Lipid Carriers Using Full Factorial Design. Pharmaceutics 12, 682 (2020).

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III. **Eszter, L. Kiss**; Mária, Budai-Szűcs; Erzsébet, Csányi: Improving the ophthalmic bioavailability of steroidal anti-inflammatory drugs, I. Symposium of Young Researchers on Pharmaceutical Technology, Biotechnology and Regulatory Science, Szeged, Hungary, 2019.01.31. (Verbal presentartion)

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