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Summary of Ph.D. thesis

FORMULATION OF MELOXICAM POTASSIUM-CONTAINING, CYCLODEXTRIN-BASED NASAL POWDERS VIA SPRAY DRYING

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2025

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1. INTRODUCTION

Nasal drug delivery has become a promising non-invasive route due to its high vascularization, rapid onset of action, and potential for avoiding first-pass metabolism. This route can facilitate direct drug delivery to the systemic circulation enhancing therapeutic efficacy. However, the nasal route faces challenges such as the inefficient permeation of drugs through the nasal mucosa and the rapid clearance of the formulations from the nasal cavity due to the mucociliary clearance. These challenges necessitate innovative strategies.

Cyclodextrins have gained considerable attention in the pharmaceutical industry. Their ability to form inclusion complexes with a variety of drugs enhances solubility and stability. Additionally, they can interact with biological membranes disrupting the cell membrane fluidity and the tight junctions between epithelial cells, enhancing the permeation.

While cyclodextrins enhance permeation, the rapid clearance of formulations from the nasal cavity remains a significant challenge. To address this, the incorporation of water-soluble polymers was explored. These polymers can increase the viscosity of the formulation, thereby prolonging the residence time in the nasal cavity.

Nasal powders produced by spray drying may also provide prolonged residence time in the nasal cavity compared to liquid formulations due to their better adhesion, thus reduced mucociliary removal. Furthermore, preservatives are unnecessary in the formulation process, and the fine particle size achieved through spray drying ensures optimal deposition and absorption of the drug within the nasal cavity. Nasal systemic delivery of non-steroidal anti-inflammatory drugs (NSAIDs) is a less explored area; however, they could serve as quick pain relievers or could be adjuncts to opioid therapy.

To the best of our knowledge, no study is available in the literature, where spray-dried cyclodextrin-based nasal powders are investigated in combination with water soluble polymers in order to deliver an NSAID to the systemic circulation through the nasal route. This Ph.D. work aims to study the applicability of different cyclodextrins in combination with water-soluble polymers with a purpose to contribute to the advancement of solid nasal drug delivery systems.

Abbreviations: *API – Active pharmaceutical ingredient; ALI – Air-liquid interface; BCD – β-cyclodextrin; CD – Cyclodextrin; CNS – Central nervous system; COX – Cyclooxygenase; DSC – Differential scanning calorimetry; FT-IR – Fourier transform infrared spectroscopy; HA – Sodium hyaluronate; HPBCD – Hydroxypropyl β-cyclodextrin; HPLC – High performance liquid chromatography; HPMC – Hydroxypropyl methyl cellulose; ICH – International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; LDH – Lactate dehydrogenase; MTS – 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; MXP – Meloxicam potassium; NSAID – Non-steroidal anti-inflammatory drug; PBS – Phosphate buffer; PM – Physical mixture; PVA – Polyvinyl alcohol; QABCD – Quaternary ammonium β-cyclodextrin; SBECD – Sulfobutylether β-cyclodextrin sodium salt; SEM – Scanning electron microscopy; SNES – Simulated nasal electrolyte solution; XRPD – X-ray powder diffractometry*

2. AIMS OF THE WORK

This PhD work aimed to produce solid nasal dosage forms containing an NSAID, meloxicam potassium (MXP) monohydrate with different excipients. The following steps were planned to conduct during our work:

I. An extensive review of the literature was proposed to determine the key characteristics of solid nasal dosage forms, their preparation techniques and the requirements of the applicable excipients.

II. After studying the literature, nano spray drying was chosen as the preparation method with the aim of preparing particles with a diameter of <5 μm. An essential physico-chemical characterization of the prepared formulations was planned to carry out. The particle size and morphology, crystallinity, thermal behavior of the samples and the potentially formed secondary interactions were aimed to be observed to reveal the effect of the preparation procedure on the properties of the powders.

III. The *in vitro* properties, like mucoadhesivity, dissolution and permeation rate of the samples were planned to study to observe the effect of the applied excipients and to choose the best-performing formulations for further stability and cell culture measurements.

IV. Our aim was also to screen the selected formulations with accelerated stability tests according to the International Council for Harmonization (ICH) Q1A guideline. The results of the stability test together with the *in vitro* measurements were intended to be the basis of the sample selection for further nasally relevant cell culture measurements.

V. Besides, our goal was to determine the non-toxic concentrations of the applied cyclodextrins and raw MXP. Furthermore, the non-toxic concentrations of the selected formulations were intended to be evaluated to be able to proceed with the permeation studies safely.

VI. Applying RPMI 2650 cells, the MXP permeation from the raw API and the selected formulations were planned to be examined to observe if the cyclodextrins had any permeation enhancing effect.

VII. Lastly, the effect of the incorporated excipients – different cyclodextrins and water-soluble polymers – on the properties of the formulations and on their nasal applicability were intended to be evaluated.

Ultimately, the primary objective of this Ph.D. work was to contribute to the development of future nasal powder products by collecting novel results on such formulations containing MXP, cyclodextrins and water-soluble polymers.

3. MATERIALS AND METHODS

3.1. Materials

Meloxicam potassium (MXP) monohydrate was used as the API in this work and was obtained from Egis Ltd. (Budapest, Hungary). 4 different CDs, namely β-cyclodextrin (BCD), (2-hydroxy)-propyl-β-cyclodextrin (HPBCD), sulfobutylated-beta-cyclodextrin sodium salt (SBECD, DS~6) and (2-hydroxy-3-N,N,N-trimethylamino)propyl-beta-cyclodextrin chloride (QABCD, DS~2.7) were all obtained from CycloLab Ltd. (Budapest, Hungary). The two polymers, hyaluronic acid sodium salt (HA) was from Contipro Biotech (Dolní Dobrouč, Czech Republic) and (polyvinyl)alcohol (PVA) was from Sigma Aldrich (Sigma Aldrich Co. LLC, St. Louis, MO, USA).

3.2. Methods

3.2.1. Preparation method of the spray-dried samples

BÜCHI Nano Spray Drier B-90 HP (BÜCHI Labortechnik AG, Flawil, Switzerland) was used for the preparation of the samples. The feeding solutions for the polymer-free formulations consisted of 1:1 molar ratio of MXP and CD (BCD/HPBCD/SBECD/QABCD) dissolved in distilled water. For the polymer-containing formulations, the solutions additionally included 1 mg/mL PVA or 0.5 mg/mL HA. The following parameters were applied for the spray drying: inlet air temperature: 80 °C, compressed air flow: 130 $L \cdot h^{-1}$, pump rate: 20%, aspirator capacity: 100%.

Physical mixtures (PM) were prepared as reference samples, maintaining the same ratios as those in the spray-dried solutions. The components were blended using a Turbula mixer (Turbula WAB, Systems Schatz, Muttenz, Switzerland) at 50 rpm for 10 minutes.

3.2.2. Physico-chemical characterization

3.2.2.1. Laser diffraction

The particle size distribution of the spray-dried products was characterized using a Malvern laser diffractometer (Malvern Mastersizer Scirocco 2000; Malvern Instruments Ltd., Worcestershire, UK). Air was used as the dispersion medium during the dry analysis and its pressure was set to 2 bar. Approximately 1 g of each sample was tested, and all samples were measured in triplicate. The particle size was characterized by the D(0.5) value, indicating the diameters below which 50% of the particles by volume are found.

3.2.2.2. Scanning electron microscopy (SEM)

SEM analysis (Hitachi S4700, Hitachi Scientific Ltd., Tokyo, Japan) was conducted to examine the surface characteristics, shape, and size of the prepared particles. The powders were coated with gold-palladium by a sputter coater and analyzed at 10 kV and 10 μA under an argon atmosphere.

3.2.2.3. X-ray powder diffractometry (XRPD)

X-ray diffractograms of the PMs and spray-dried samples were recorded using a BRUKER D8 Advance diffractometer (Bruker AXS GmbH, Karlsruhe, Germany) with Cu Kα radiation (λ = 1.5406 Å), operating at a tube voltage of 40 kV and a tube current of 40 mA. Data were collected over an angular range of 3–40° 2θ, with a step time of 0.1 seconds and a step size of 0.007°. Signal detection was performed with a VÅNTEC-1 detector. The manipulations, including Kα2 stripping, background removal, and smoothing, were conducted using DIFRAC.EVA software.

3.2.2.4. Differential Scanning Calorimetry (DSC)

The thermal behavior of the samples was analysed using a Mettler Toledo DSC 821^e differential scanning calorimeter (Mettler Inc., Schwerzenbach, Switzerland). Samples weighing 2-5 mg were placed in perforated aluminum pans and heated from 25 to 300 °C at a rate of 10 °C·min^{-1} under a constant argon flow of 150 mL·min^{-1}. The recorded DSC curves were analysed using the STAR^e thermal analysis software (Mettler Toledo; Mettler Inc., Schwerzenbach, Switzerland).

3.2.2.5. Fourier transform infrared spectroscopy (FT-IR)

FT-IR measurements were used to detect the interactions between MXP and the excipients. Individual compounds and spray dried products were measured and compared. The spectra were recorded with an AVATAR330 spectrometer (Thermo Nicolet, Unicam Hungary Ltd., Budapest, Hungary), the interval was in the range of 400–4000 cm^{-1}, at an optical resolution of 2 cm^{-1}. Pastilles prepared from the samples with 0.15 g KBr were investigated. SpectraGryph optical spectroscopy software was used to evaluate and present the detected spectra.

3.2.3. *In vitro* measurements

3.2.3.1. Texture analyser

The adhesive properties of the formulations were investigated with a TA-XT Plus Texture Analyser (Metron Ltd., Budapest) instrument utilizing a cylinder probe with a 1 cm diameter and a 5 kg load cell. Before the measurement, 95±5 mg of the samples was compressed into pastilles with a Specac hydraulic press (Specac Inc., Orpington, UK) applying a pressing force of 10 kN for 10 seconds with a pressing diameter of 13 mm. The pastilles were attached to the cylinder probe and brought into contact with the artificial mucosa layer for 3 minutes at a preload of 2500 mN. After that, the cylinder probe was raised to detach the sample from the filter paper at a speed of 2.5 mm·min^{-1}, while a force-distance curve was recorded determining the maximum detachment force and work of adhesion. The latter was calculated as the area under the "force vs. distance" curve. For the artificial mucosa, a filter paper was wetted with 40 µl of an 8% w/w mucin dispersion prepared with simulated nasal electrolyte

solution (SNES). Control measurements were performed using SNES-wetted filter papers to observe the behavior of the powders without mucin. For statistical analysis, one-way ANOVA with Tukey's multiple comparison test was applied using Minitab statistical software. A level of $p \leq 0.05$ was considered as significant.

3.2.3.2. Drug release study modelling nasal conditions

The drug release from the samples was monitored in-line over a period of 5 minutes at a wavelength of 364 nm by adding powders containing 0.75 mg of the API into 50 mL of SNES. The system was maintained at 32 ± 0.5 °C with a constant stirring of 100 rpm. The results were quantified using an AvaLight DH-S-BAL spectrophotometer (Avantes, Apeldoorn, Netherlands) connected to an AvaSpec-2048L transmission immersion probe (Avantes) via an optical fiber.

3.2.3.3. Permeation study through an artificial membrane

A modified horizontal diffusion cell was used to test the *in vitro* diffusion of MXP under nasal conditions and to identify promising formulations for further stability and cell line investigations. The device consisted of two chambers – one for the donor phase and one for the acceptor phase – separated by an artificial membrane. The donor phase contained 9 mL of SNES, representing the nasal fluid, while the acceptor phase contained 9 mL of phosphate buffer solution (pH 7.4) to simulate blood pH. Both phases were maintained at 32 °C (Thermo Haake C10-P5, Sigma Aldrich Co.) and stirred at 300 rpm using a magnetic stirrer to simulate the airflow and the cilia. To simulate the lipophilic mucosa, a Whatman™ regenerated cellulose membrane filter with 0.45 μm pores was soaked in isopropyl myristate for 30 minutes before the test. Samples containing 7.5 mg of MXP were added to the donor phase at the beginning of the study. Aliquots were collected at predetermined intervals from the acceptor phase and the cumulative amount of permeated MXP was quantified. For statistical analysis, one-way ANOVA with Fisher's multiple comparison test was applied using Minitab statistical software. A level of $p \leq 0.05$ was considered as significant.

3.2.4. Accelerated stability test

The stability of the selected formulations was studied under the accelerated stability test conditions according to the ICH Q1A (R2) guideline. The powders were filled into hydroxypropyl-methyl cellulose (HPMC) capsules and placed into a Binder KBF 240 (Binder GmbH Tuttlingen, Germany) constant-climate chamber without secondary packaging maintaining 40 ± 2 °C and 75 ± 5% RH. The formulations were re-examined after 1 month in terms of morphology, crystallinity and drug content. The results of the stability test contributed to the selection among the formulations for further cell culture measurements.

3.2.5. Cell-based measurements

3.2.5.1. Cytotoxicity on RPMI 2650 cells

To evaluate the viability of RPMI 2650 cells, the CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay (Promega GmbH, Mannheim, Germany) was employed. Cells were cultured and seeded into 96-well plates at a density of 5×104 cells per well and maintained until confluence. Test samples - including raw MXP, BCD, SBECD, QABCD alone and BCD-MXP-PVA-SD, SBECD-MXP-PVA-SD, QABCD-MXP-PVA-SD formulations - were added at various concentrations: the CDs in 5, 2.5 and 1 mM concentration, raw MXP in 125, 95, 65, and 25 μg/mL, and the formulations in 125, 95 and 65 μg/mL MXP concentration. The cells were incubated with the solutions for 1 hour at 37 °C. After treatment, the cells were washed with Hank's Balanced Salt Solution and incubated with the MTS reagent for 2 hours at 37 °C. Absorbance was measured at 491 nm using a UV/VIS microplate reader.

For assessment of membrane integrity, lactate dehydrogenase (LDH) release was quantified using the CytoTox-ONE™ Homogeneous Membrane Integrity Assay (Promega GmbH, Mannheim, Germany). Fluorescence was recorded at an excitation wavelength of 560 nm (emission wavelength: 590 nm) using a plate reader. Untreated cells served as negative controls, and lysed cells served as positive controls. Background was subtracted from all measured values to ensure accuracy. For statistical analysis, one-way ANOVA with Dunnett's test was applied using Minitab statistical software. A level of $p \leq 0.05$ was considered as significant.

3.2.5.2. Drug permeation through nasal RPMI 2650 cell model

The diffusion of MXP from the selected PVA-containing formulations was studied on an RPMI 2650 epithelial cell model cultured under air-liquid interface (ALI). For the model, RPMI 2650 cells at a density of 50,000 cells per 0.33 cm² were seeded onto Transwell™ filter inserts (3 μm pore size, polycarbonate). The cells were cultured in a liquid-covered state for 8 days with refreshing the medium (MEM supplemented with 10% FBS, 1% Penstrep, and 1% non-essential amino acids) every 2–3 days. After 8 days, the inserts were raised to an ALI and cultured for an additional 2-3 weeks to ensure full differentiation of the cells.

For the test, BCD-MXP-PVA-SD, SBECD-MXP-PVA-SD and QABCD-MXP-PVA-SD samples were dissolved in phosphate buffer (PBS, pH 7.4) to reach a non-toxic MXP concentration. The same concentration of raw MXP in PBS (pH 7.4) was applied as a control. 600 μL of PBS (pH 7.4) was added to the basolateral compartment, and to start the permeation studies, 100 μL of the sample solutions was added to the apical side of the Transwell™ insert. Samples were taken from the basolateral side at specific time points (5,

10, 15, 30, 45, and 60 minutes) to measure the permeation of MXP across the epithelial layer. The solutions from the apical side were also collected to assess the decrease in API content after 60 minutes. The HPLC method described in section 3.2.6. was used for the determination of MXP-content. For statistical analysis, one-way ANOVA with Fisher's multiple comparison test was applied using Minitab statistical software. A level of $p \leq 0.05$ was considered as significant.

3.2.6. MXP quantification by high performance liquid chromatography (HPLC)

An Agilent 1260 HPLC system (Agilent Technologies, San Diego, United States) was used to quantify the MXP amount in the samples. The mobile phase consisted of PBS (pH=2.8) and methanol in a ratio of 42:58 (% v/v). A Kinetex® EVO 5 μm C18 100 Å column (150 x 4.6 mm, Phenomenex, Torrance, CA, USA) was used for the separation. The injection volume for the samples was 10 μL and the separation was performed with an isocratic method, with the flow rate of 1 $mL \cdot min^{-1}$ for 6 minutes at 30 °C. MXP was quantified at 364 nm using a diode array detector. Data were evaluated with ChemStation B.04.03. software (Agilent Technologies, Santa Clara, CA, USA).

4. RESULTS AND DISCUSSION

4.1 Physico-chemical characterization of the prepared samples

4.1.1. Particle size and morphology

Laser scattering was applied for the determination of the particle size. The average particle size was between 1.89 and 3.05 μm (Table 1.).

Table 1. D(0.5) values of the samples

Sample	D(0.5) (μm)
BCD-MXP-SD	1.89 ± 0.09
BCD-MXP-PVA-SD	1.97 ± 0.06
HPBCD-MXP-SD	2.72±0.09
HPBCD-MXP-PVA-SD	2.58±0.21
HPBCD-MXP-HA-SD	3.05±0.15
SBECD-MXP-SD	2.21 ± 0.06
SBECD-MXP-PVA-SD	2.14 ± 0.03
QABCD-MXP-SD	2.10 ± 0.03
QABCD-MXP-PVA-SD	1.89 ± 0.06

Based on the SEM analysis, the spray dried particles consistently demonstrated a uniform, spherical shape and smooth surface characteristics, some submicron-sized particles were also noticeable on them.

4.1.2. Crystallinity of the samples

The crystallinity of the spray-dried samples and PMs was examined by XRPD, the diffractograms are presented in Figure 1. In all PMs, the peaks of the initially crystalline MXP were detected at 2θ of 6.1, 15.5 and 24.6°. The characteristic peaks of BCD appeared at 4.5, 9.0, 10.7 and 12.5°, indicating its initially crystalline structure, as well. Considering the substituted CDs and the polymers, their diffraction pattern could not be detected suggesting their amorphous state.

In the spray dried samples, all the previously observed peak disappeared referring to the complete amorphization of every originally crystalline material, solid dispersions were formed. This probably occurred due to the preparation process: spray drying often results in the amorphization of the products.

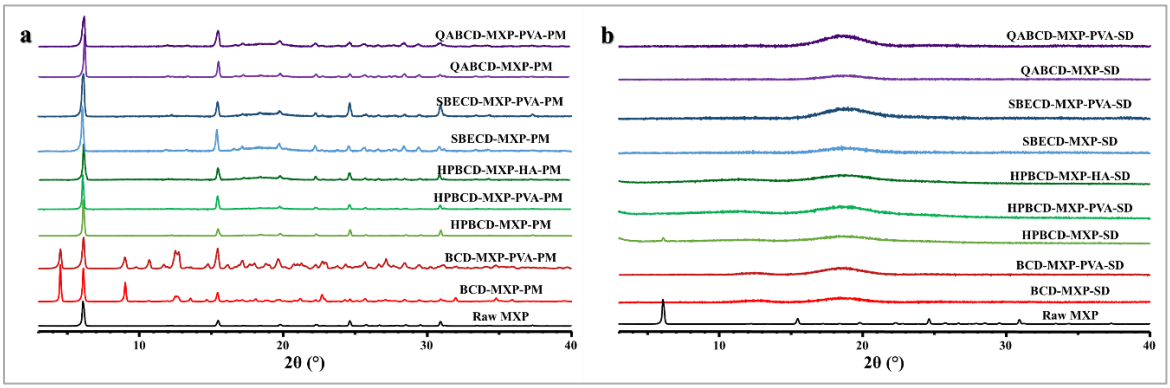

Figure 1. Diffractograms of (**a**) the PMs and (**b**) the spray-dried samples

4.1.3 Thermal behavior of the samples

DSC was used to investigate the thermal behavior of both the spray-dried samples and the PMs. The melting peak of MXP around 160-170 °C in the PMs disappeared from the thermograms of the spray-dried samples and the peak of its decomposition also shifted, broadened and diminished indicating the amorphization of MXP in the formulations. The results of the DSC corresponded to that of the XRPD.

4.1.4. Secondary interactions in the formulations

FT-IR measurement was implemented to study the possibly formed secondary interactions between the excipients and the drug. The individual compounds were compared to the prepared spray-dried samples, the spectra are presented in Figure 2.

MXP exhibited the typical peak of C=O from the amide bond at 1618 cm^{-1}. In the spectra of the CDs, the peak assigned to the O–H stretching was observed in the 3790–3000 cm^{-1} wavenumber range and the peak of O–H bending appeared at 1640 cm^{-1}, 1653 cm^{-1}, 1647 cm^{-1} and 1598 cm^{-1} for BCD, HPBCD, SBECD and QABCD, respectively.

In the spray-dried formulations, the main event that could be observed was the appearance of a merged, shifted sharp peak in the 1650–1550 cm^{-1} wavenumber range. All BCD- and

HPBCD-containing formulations exhibited it at 1616 cm^{-1} and the SBECD-containing formulations at 1623 cm^{-1}. This indicates the potential engagement of the API and the excipients in hydrogen bonding. The shifting occurred regardless of the presence of PVA and HA, their effect might have been shaded because of the low polymer-content of the samples.

In the QABCD-containing formulations, no change was observed in the spectra indicating the absence of secondary interactions; however, this measurement method is not able to detect the possibly formed electrostatic interaction between MXP and QABCD in the formulation.

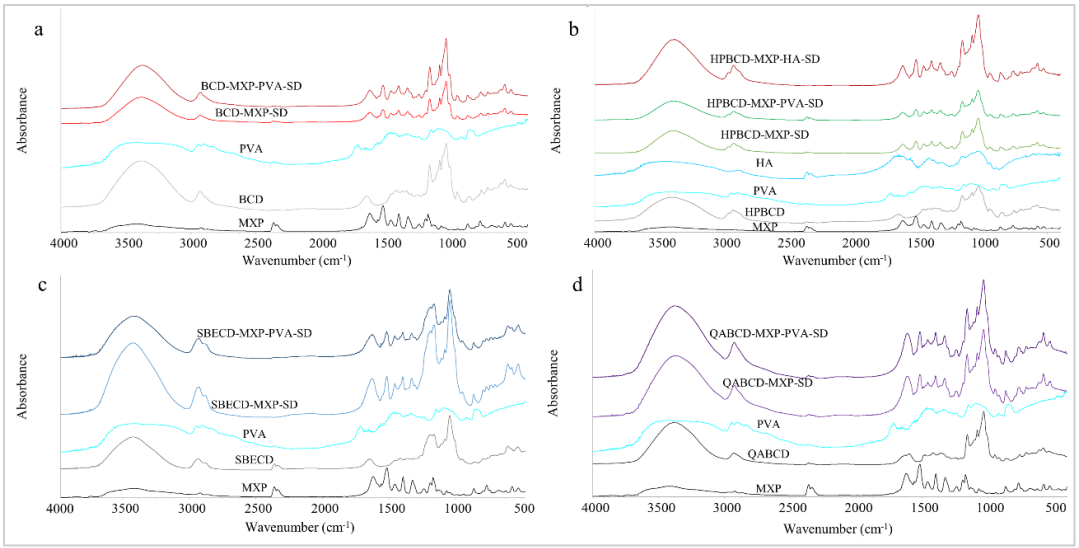

Figure 2. FT-IR spectra of the individual compounds and (**a**) BCD-related samples, (**b**) HPBCD-related samples, (**c**) SBECD-related samples and (**d**) QABCD-related samples

4.2 *In vitro* measurements

4.2.1. *In vitro* mucoadhesion test

A texture analyser was applied to mimic and test the adhesion of the powders to the nasal mucosal surface. Conclusions were made based on the recorded maximum detachment force and work of adhesion values, which are presented in Figure 3.

Considering maximum detachment force, significantly higher values were detected for each type of sample when contacting mucin compared to SNES. Furthermore, in the presence of mucin, every CD-based sample was significantly different from raw MXP except for BCD-MXP-SD. The polymer content did not affect the peak force, neither PVA nor HA showed a significant effect. Thus, the presence of the CDs was necessary for the formation of these interactions except for BCD, while the same could not be determined with certainty for the polymers.

As for the work of adhesion, in most of the cases no significant difference was found between the mucin and SNES contacting samples. However, the measured values were similarly high

to a mucoadhesive cysteine-chitosan conjugate prepared and examined for nasal application by Kiss et al. Work of adhesion is considered to reflect both the physical interpenetration and entanglement of the polymer chains and mucin along with the strength of secondary interactions. In our case, the reason of the detected high work of adhesion values was probably the water uptake of the samples. Presumably, after the wetting and swelling of the powders, the liquid was absorbed quickly through the capillaries of the pastilles when contacting the filter papers. This process likely resulted in the dehydration of the artificial mucosal surface thereby creating a strong physical adhesion irrespective of mucin's presence. Our observation suggests that the samples may initially display significant adhesive strength to the nasal mucosa prior to their dissolution.

In summary, the presence of the CDs – except for BCD – was essential for establishing secondary interactions with mucin, moreover, the powders may exhibit a strong adhesion upon initial contact with the mucosa. While this measurement did not confirm the advantageous role of the polymers, their inclusion in nasal formulations could provide enhanced viscosity at their application site potentially decelerating the mucociliary clearance.

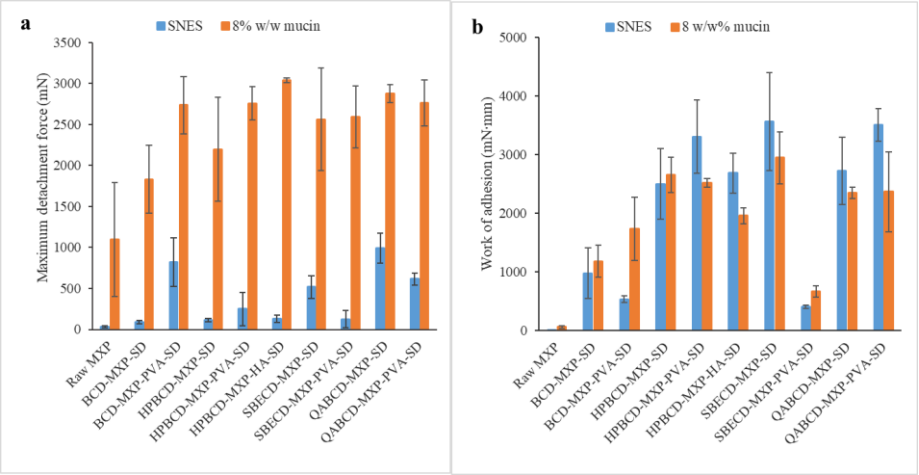

Figure 3. (**a**) Maximum detachment force and (**b**) work of adhesion values

4.2.2 *In vitro* dissolution study

The MXP dissolution from the samples was monitored in-line applying nasal conditions, the results are presented in Figure 4. Raw MXP, used as a reference, dissolved significantly slower than any other formulation, only 46% of it was detected in the solution after 5 min. In contrast, all spray-dried formulations achieved nearly complete dissolution within the first minute. The applied polymers did not have a noticeable impact on the dissolution rate.

The experienced rapid API dissolution can be attributed to multiple contributing factors, including particle size reduction, amorphization of MXP in the products and the presence of highly water-soluble CDs, which could improve the wettability of the formulations. Since drug absorption through the nasal mucosa requires the API to be dissolved in the mucus,

faster dissolution may lead to enhanced bioavailability because of the higher concentration gradient.

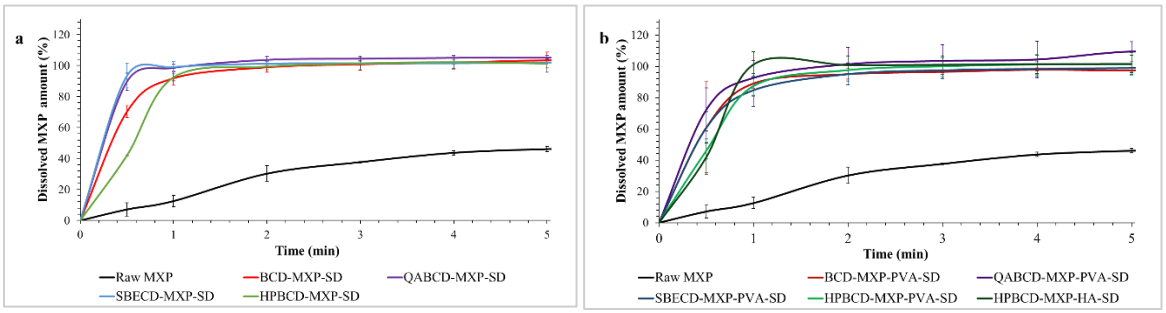

Figure 4. *In vitro* MXP release from the (**a**) polymer-free and (**b**) polymer-containing formulations

4.2.3 *In vitro* permeation studies applying an artificial mucosa

A horizontal diffusion model with an artificial mucosa was applied to test the *in vitro* diffusion of the samples, Figure 5. presents the results. Raw MXP was selected as a reference compound, from which 54.6 $\mu g \cdot cm^{-2}$ diffused to the acceptor phase within the 1-hour period. Among the polymer-free samples, the highest extent of drug permeation was observed from the cationic QABCD-based formulation, with a significantly greater amount compared to raw MXP and the other three formulations. Notably, BCD-MXP-SD, HPBCD-MXP-SD and SBECD-MXP-SD showed similar drug diffusion values.

Considering the polymer containing samples, HA-content seemed to be favorable in the HPBCD-based sample, it exhibited a significantly higher permeation rate compared to raw MXP and the polymer-free samples, but significantly lower than the PVA-containing samples.

In the presence of PVA, all CD-based formulations demonstrated markedly enhanced permeation rates. QABCD-MXP-PVA-SD exhibited an outstanding diffusion rate. The BCD, HPBCD- and SBECD-based PVA-containing formulations were nearly equivalent to the PVA-free QABCD-MXP-SD formulation. These findings highlight the influence of both the cyclodextrin's type and the presence of PVA on API permeation.

QABCD significantly enhanced MXP permeation relative to BCD, HPBCD and SBECD. We hypothesize that electrostatic interactions contributed to the improved permeation: upon dissolution, MXP dissociates into potassium ions and anionic meloxicam, which can interact favorably with the cationic moieties of QABCD. This interaction likely plays a key role in the observed increase in diffusion.

Overall, PVA consistently exhibited beneficial effect on the permeated drug quantity under simulated nasal conditions, it outperformed HA.

For further experiments, the PVA-containing samples were selected to gain more insight into their stability and the potential permeation-enhancing properties of the CDs on a nasal cell line.

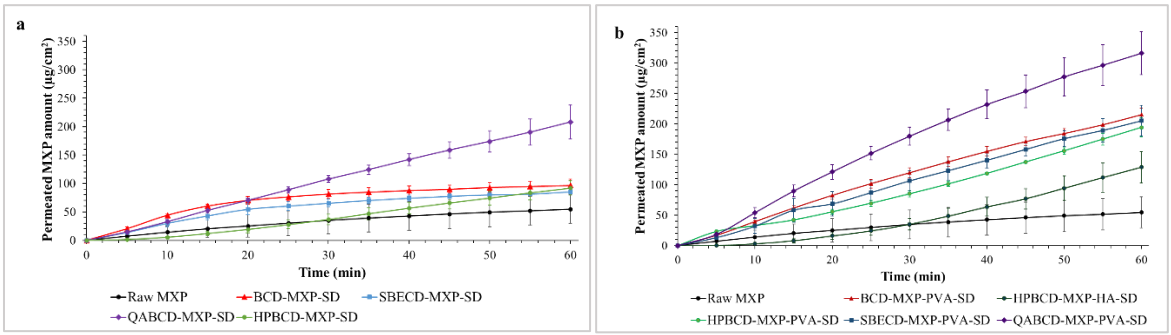

Figure 5. *In vitro* MXP diffusion from the (**a**) polymer-free and (**b**) polymer-containing formulations

4.3. Accelerated stability testing of the PVA-containing samples

4.3.1. Morphology of the particles

The morphology was observed applying SEM, the images are shown in Figure 6. As stated before, spray drying resulted in spherical, smooth surfaced particles with an average particle size around 2 μm. After 1 month of storage, differences in the morphology were detected. The BCD-based formulation did not show any obvious change, while aggregate formation between the particles of HPBCD-MXP-PVA-SD was clearly visible. The most drastic change in the morphology of the particles was observed in the two charged CD-based formulations: the individual particles disappeared, merged, glass-like blocks were formed in both cases. The exhibited changes were most likely a consequence of the highly humid environment.

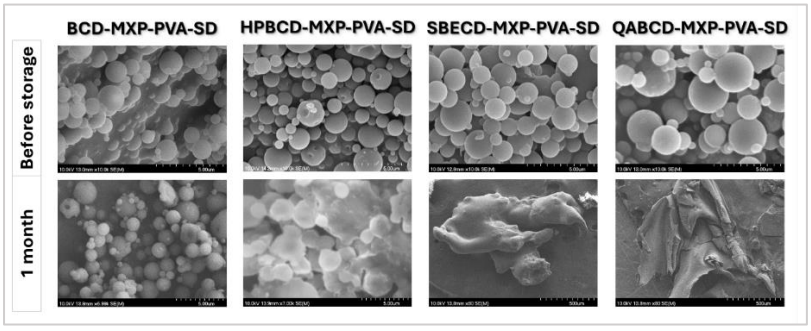

Figure 6. SEM images of the PVA-containing samples from before storage and after 1 month

4.3.2. Physico-chemical stability

Based on the diffractograms of the samples, as a result of spray drying, the initial formulations were amorphous, and after 1 month of storage, neither of the samples showed any signs of recrystallization. This could be attributed to both the presence of PVA and the amorphous CDs.

The thermal behavior of the samples was also studied, the two neutral CD-based samples did not show any change in their thermograms. However, new endothermic peaks appeared after 1 month in the thermograms of the SBECD- and QABCD-based samples probably due to the water uptake.

4.3.3. Drug content change of the samples

The drug content of the samples was also studied during the stability test, the statistical comparison of the results was carried out with 2-sample t-tests. After 1 month, there was a significant decrease in the drug content of BCD-MXP-PVA-SD and SBECD-MXP-PVA-SD sample comparing them to their "before storage" state. Their drug content was above the 5% acceptance limit defined by the ICH Q1A (R2). The experienced decrease was most likely due to the degradation of the API.

4.4. Cell-based measurements

4.4.1. In vitro toxicity on RPMI 2650 cells

CDs and MXP alone as well as BCD-MXP-PVA-SD, SBECD-MXP-PVA-SD and QABCD-MXP-PVA-SD were selected for further cell-based measurements. Their non-toxic concentrations were determined based on their effects on membrane integrity and cell viability after 1 h of incubation using nasal RPMI 2650 cells.

As shown in Figure 8., CDs alone did not influence the cell viability, while they showed a concentration-dependent effect on the membrane integrity. BCD did not seem to disturb the membrane integrity in the tested concentration range, while SBECD and QABCD caused a significant increase in the LDH release compared to cell control above 1 mM and 2.5 mM concentrations, respectively. According to our findings, BCD can be considered more tolerable to RPMI 2650 cells than QABCD and SBECD. The cytotoxic effect of these cyclodextrins had not been studied yet on RPMI 2650 cells except for BCD. According to the literature, the toxicity of CDs correlates with their cholesterol extraction capacity.

Regarding raw MXP and the tested PVA-containing formulations, neither of them caused a significant increase in LDH release over the concentration range tested compared to the cell control. In contrast, cell viability assays indicated a significant decrease testing raw MXP solutions in the range of 125-25 μg/ml except for 25 μg/ml, but only the 125 μg/ml solution resulted in decreasing viability to 68%. For the spray-dried formulations, only the 125 μg/ml MXP-containing solution of BCD-MXP-PVA-SD resulted in a significant reduction in viability to 72±2 %, neither SBECD-MXP-PVA-SD, nor QABCD-MXP-PVA-SD approached the viability threshold of 70 %. CDs can potentially play a protecting role against the toxic effect of APIs.

For further experiment, to ensure that the results of the permeability study are not affected by the toxicity of the formulations so that the results are comparable, a concentration of 95 μg/ml of MXP was chosen.

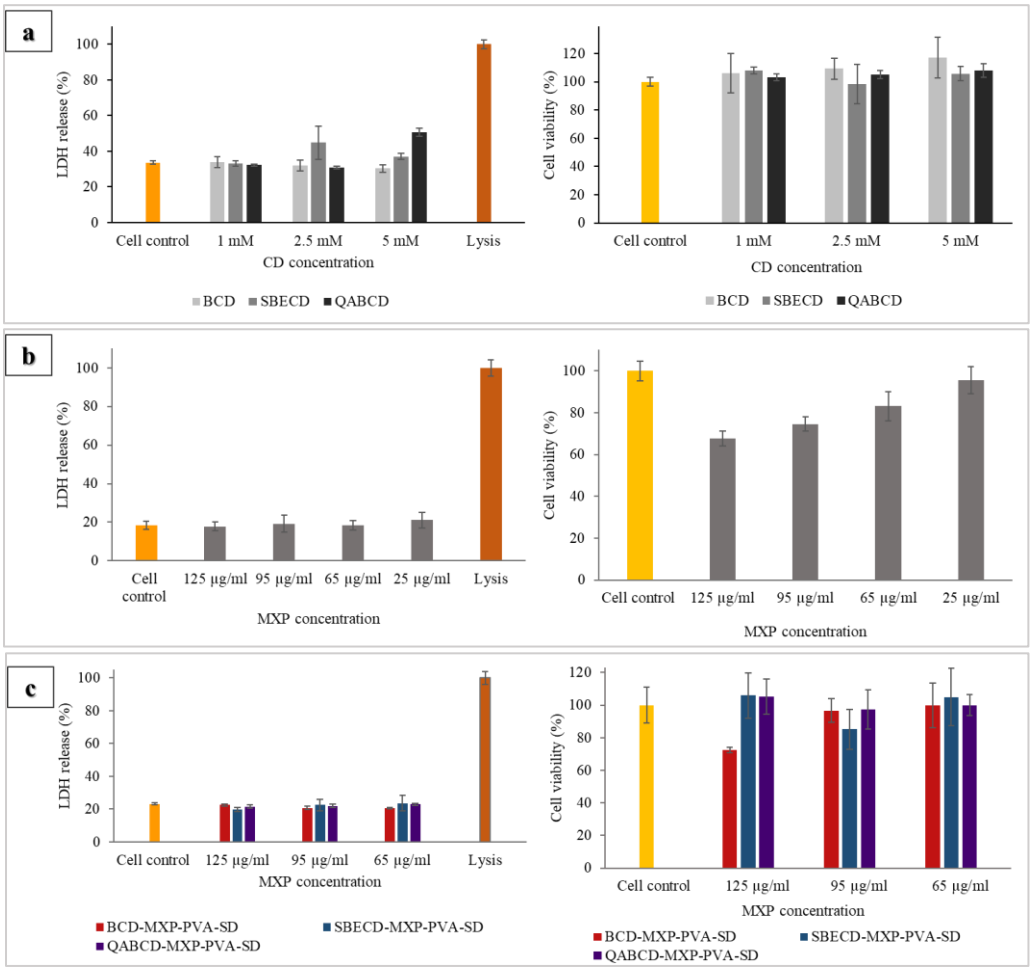

Figure 8. Effect on the LDH release and cell viability (%) of (**a**) CDs, (**b**) raw MXP and (**c**) the formulations

4.4.2. In vitro permeation studies applying RPMI 2650 nasal cells

For the permeation study, 95 µg/ml MXP concentration solutions were applied for the raw API as well as the PVA-containing formulations. The results are presented in Figure 9. and Table 2.

Among the samples, SBECD-MXP-PVA-SD exhibited a permeation of 4.5 µg/cm^2, which was significantly higher than all the other formulations and raw MXP, which showed comparable, lower permeation rates. After 60 mins, the highest drug concentration in the apical compartment was found for raw MXP. The QABCD-based and BCD-based samples demonstrated significant changes compared to raw MXP. SBECD-containing formulation resulted in the lowest drug concentration, significantly differing from all the other samples. It can be concluded that only the presence of the negatively charged SBECD resulted in enhanced permeation of the drug on the tested cell line during the experimental period. CDs are known to have permeation enhancing features due to combined effects of two factors: improving the solubility of drugs making them accessible for absorption and interacting with biological membranes. In our case, the solubility enhancing effect of SBECD was not

proven, most likely due to electrostatic repulsive forces between the negatively charged CD and meloxicam anion. However, SBECD could still have affected the cells, which proves that the presence of CDs may be favorable not only for drugs with low water-solubility.

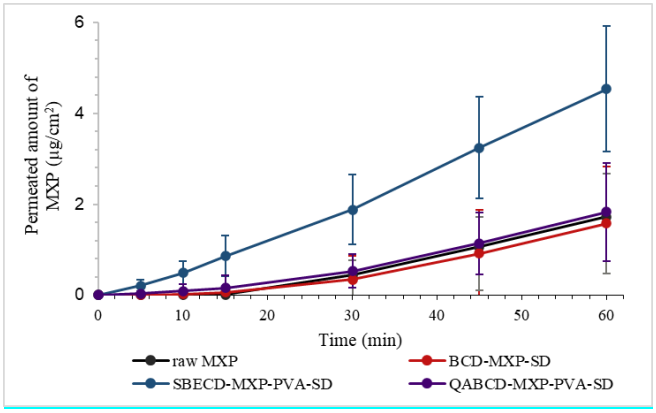

Figure 9. Permeated amount of MXP through RPMI 2650 cells

Table 2. MXP amount in the apical and basolteral compartment after 1 hour

	MXP amount in apical compartment after 60 min (%)	Permeated MXP amount after 60 min (%)
Raw MXP	79.6 ± 1.2	5.4 ± 2.9
BCD-MXP-PVA-SD	71.5 ± 1.0	5.5 ± 3.8
SBECD-MXP-PVA-SD	63.3 ± 0.6	15.8 ± 4.8
QABCD-MXP-PVA-SD	74.7 ± 4.8	6.4 ± 3.7

5. CONCLUSIONS

The findings of this PhD work, which aimed to prepare MXP-containing solid nasal dosage forms are summarized in the following points:

I. Based on the literature review, we decided to apply different BCDs and water-soluble polymers as excipients and nano spray drying as preparation technique. Nasal powders were aimed to be prepared containing a patented but so far not marketed NSAID, MXP.

II. With the applied preparation method, amorphous microparticles were prepared with spherical morphology and smooth surface characteristics. The originally crystalline materials, including MXP were amorphized as a consequence of the preparation method. The formation of secondary interactions between the CDs and MXP was confirmed except for the QABCD-based samples; however, the possibly formed electrostatic interaction between meloxicam anion and cationic QABCD was not detectable with FT-IR.

III. During the *in vitro* mucoadhesion test, the following phenomena were observed. (1) The maximum detachment force was significantly higher in every case compared to raw MXP when samples contacted with mucin, except for BCD. This suggests that the presence of CDs contributed to the formation of the secondary interactions between the samples and mucin.

(2) The powders presented strong adhesion which could have occurred due to their dehydrating effect potentially leading to strong *in vivo* adhesion until their dissolution. (3) The type of the cyclodextrins did not seem to affect the adhesion of the formulations based on this measurement. (4) Neither the formed secondary interactions, nor the strength of the adhesion was influenced by the presence of the polymers evidently.

The *in vitro* dissolution of MXP under nasal conditions was accelerated significantly. The complete release of the API was observed in 1 min from all the samples unlike the raw MXP probably due to amorphous solid dispersions and the low particle size. The combination of this preparation method with the applied excipients can be considered highly suitable to prepare nasal powders with fast drug release.

The drug release was not a rate determining step in the *in vitro* permeation of MXP through the artificial mucosa, the type of the CD as well as the type of the polymer influenced the permeation rate. The cationic QABCD outperformed all the other samples in the presence of PVA. Even without PVA, it exhibited a similar permeation rate to the other, PVA-containing samples presumably due to formed electrostatic attracting forces between meloxicam anion and cationic QABCD. HA also improved the permeation rate of MXP, although it significantly underperformed PVA, PVA improved the passive diffusion of MXP from all samples. Thus, PVA-containing samples were selected for further stability studies.

IV. The accelerated stability screening provided mixed results and conclusions; the choice of the best performing formulation was not possible. In terms of morphology, BCD-MXP-PVA-SD provided the best results. The amorphous state was maintained for all samples, the solid dispersion was stabilized by PVA and the amorphous CDs, while a decrease in drug content was detected for BCD- and SBECD-based samples. The observed changes can be attributed to the effect of the conditions of the study, the high tendency to absorb water was proven for all the substituted CD-based samples. Cell-based measurements were proceeded with BCD-MXP-PVA-SD, SBECD-MXP-PVA-SD and QABCD-MXP-PVA-SD formulations.

V. The toxicity studies, which were carried out for the first time with the charged CDs and MXP on RPMI 2650 cells, revealed that while CDs affected the LDH release of the cells, MXP impacted the cell viability in a concentration dependent manner. BCD did not cause any significant change in the LDH release comparing it to the cell control in the examined concentration range, while the 2.5 mM concentration of SBECD and 5 mM concentration of QABCD resulted in a significant increase. For raw MXP, the cell viability percentage was lower than 70% for the 125 µg/ml concentration solution which is considered as the acceptance limit of non-cytotoxic effect.

The formulations were tested based on the MXP concentration of their solutions. Only the 125 μg/ml MXP concentration solution of BCD-MXP-PVA-SD caused a significant decrease in cell viability to 72%, all the other formulations exhibited higher viability values, which suggests that the applied CDs might have had a protecting effect on the cells at higher MXP concentrations. To ensure that the further permeation study results were not affected by the toxicity of the formulations, the 95 μg/ml MXP concentration-solutions were tested.

VI. For the permeation study of MXP, ALI-cultured RPMI 2650 cells were applied first in the literature to examine the potential permeation enhancing effect of the applied BCDs in nasal conditions. The accelerating effect of the CDs was evaluated taking into consideration the MXP amount in both the apical and the basolateral compartment. Based on that, it could be concluded that only SBECD had a significant impact on the diffusion of the API.

VII. To summarize the findings briefly, our preparation process yielded amorphous solid dispersions. The CDs – regardless of their type – contributed to the fast dissolution of the API together with the low particle size and the amorphous structure. Secondary interactions were detected with MXP in the formulations except for QABCD which may have formed electrostatic interaction with the drug. CDs also seemed to contribute to the formation of secondary interactions with mucin in the mucoadhesion test, but the importance of their type was not confirmed evidently. The permeation of the API through an artificial mucosa examining the polymer-free samples was improved only in the presence of QABCD. Besides, the highly water-soluble BCD derivatives tended to absorb the humidity of the environment due to which they could not maintain their morphology. The tolerability of the CDs to RPMI 2650 cells followed the order of BCD > QABCD > SBECD. The permeation enhancing effect on the cells for MXP was confirmed only for SBECD.

Neither PVA, nor HA had a proven effect on the rate of MXP dissolution or the adhesion of the samples. PVA exhibited convincing evidence for its permeation accelerating impact through the artificial mucosa, HA also improved drug diffusion, but its effect was significantly lower than PVA's.

The combination of BCDs and water-soluble polymers in nasal powder formulations for the absorption enhancement of MXP was reported first in the literature as a part of this Ph.D. work. Furthermore, the investigation of concentration-dependent cytotoxic effects of SBECD, QABCD, MXP and the mentioned formulations on RPMI 2650 nasal cell line has not been observed before. In addition to that, the permeation testing of MXP in the presence of the different BCDs and PVA was carried out first in the literature on ALI-cultured RPMI 2650 nasal cell line. Although SBECD-MXP-PVA-SD wasn't the best performing formulation in terms of drug diffusion through the artificial membrane or stability, the enhanced permeation of MXP through the nasal cell line was observed only for this sample.

The combination of SBECD and PVA provides a good base for a novel nasal powder formulation with fast drug release and potentially improved bioavailability. However, further optimization of the composition and the application of non-permeable packaging would be required to solve the stability issue.

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PRESENTATIONS RELATED TO THE THESIS

Oral presentations

1) Varga P., Ambrus R., Balla-Bartos Cs.: Nazális bevitel céljából előállított meloxikám-kálium tartalmú nanoszférák vizsgálata, MKE Kristályosítási és Gyógyszerformulálási Szakosztály 13. Kerekasztal Konferenciája (2021)

2) Varga P., Németh A.: Különböző töltéssel rendelkező β-ciklodextrin alapú meloxikám-kálium-tartalmú nazális porok vizsgálata, XV. Clauder Ottó Emlékverseny (2023)

3) Varga P., Balla-Bartos Cs., Ambrus R.: Meloxikám-kálium tartalmú nazális porok stabilitásvizsgálata, XXVI. Tavaszi Szél Konferencia, Pécs (2023)

4) Varga P., Németh A., Budai-Szűcs M., Balla-Bartos Cs., Ambrus R.: Evaluation of different β-cyclodextrins- and polymer-containing nasal powders, VI. Symposium of Young Researchers on PharmaceuticalTechnology, Biotechnology and Regulatory Science, Szeged (2024)

5) Ambrus R., Varga P., Németh A., Balla-Bartos Cs., Csóka I.: Application of Cyclodextrins in Traditional and Alternative Drug Formulations; Case Studies, Conference on Pharmacology, Pharmacokinetics & Innovation (HUPHAR 2024), Mátraháza (2024)

Poster presentations

1) Varga P., Balla-Bartos Cs., Ambrus R.: Investigation Of Meloxicam Potassium Containing Nanoparticles For Intranasal Administration, 9th BBBB International Conference on Pharmaceutical Sciences, Ljubljana (2022)

2) Motzwickler-Németh A., Varga P., Budai-Szűcs M., Csóka I. Investigation of the nasal applicability of different charged cyclodextrin-based meloxicam potassium containing particles, EUROCD 2023 7th European Cyclodextrin Conference, Budapest (2023)

3) Varga P., Balla-Bartos Cs., Ambrus R.: Stability testing ofcyclodextrin-based meloxicam potassium containing nasal powders, EUROCD 2023 7th European Cyclodextrin Conference, Budapest (2023)

4) Varga P., Balla-Bartos Cs., Ambrus R.: Accelerated stability study of differently charged β-cyclodextrin-PVA based nasal powders, Congressus Pharmaceuticus Hungaricus XVII. and EUFEPS Annual Meeting 2024, Debrecen (2024)

This work was supported by the Ministry of Human Capacities, Hungary grant TKP 2020 and 2.2.1-15-2016-00007 and GINOP-2.3.2-15-2016-00060 Projects.

NKFI OTKA K_146148 project and TKP2021-EGA-32 project with the support provided by the Ministry of Culture and Innovation of Hungary from the National Research, Development and Innovation Fund are also acknowledged.

I am also grateful for the support of ÚNKP-22-3-SZTE-163 and ÚNKP-23-3-SZTE-196 New National Excellence Programs provided by the Ministry for Culture and Innovation from the source of the National Research, Development and Innovation Fund.

I would like to express my gratitude to Prof. Dr. Ildikó Csóka for allowing me to carry out my Ph.D. studies in the Institute of Pharmaceutical Technology and Regulatory Affairs.

I am grateful for the guidance of my supervisors, Prof. Dr. Rita Ambrus and Dr. Csilla Balla-Bartos who contributed to raising the standards of my work with their valuable insights and advices. The work of my co-authors is also greatly appreciated, especially Anett Motzwickler-Németh's, who asked the right questions at the right times. Special thanks to the technicians of the Institute of Pharmaceutical Technology and Regulatory Affairs for their help. I would like to also highlight Prof. Dr. Eva Roblegg, who allowed me to join her research group for 3 months in Graz and provided insights into the mysteries of cell-culturing.

I am grateful for the friendships I have made and the opportunity to develop personally and professionally during my studies.

I greatly appreciate the emotional support of my friends and family, who were there for me the whole time and helped me through the lowest points.

Lastly, I would like to thank my husband, Dr. Tamás Kiss for his endless and unconditional love. I would not have been able to achieve the same things without his constant encouragement to step out of my comfort zone.