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Summary of Ph.D. Thesis

QUALITY BY DESIGN BASED DEVELOPMENT AND INVESTIGATION OF NASAL POLYMERIC MICELLES LOADED WITH MELOXICAM

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DEVELOPMENT AND INVESTIGATION OF NASAL POLYMERIC MICELLES LOADED WITH MELOXICAM BASED ON THE QUALITY BY DESIGN METHODOLOGY

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ABBREVIATIONS

ANOVA	Analysis of variance
AUC	Area under the curve
BBB	Blood-brain barrier
CCD	Charge-coupled device
Cl	Clearance
CMA	Critical Material Attribute
CPP	Critical Process Parameter
CQA	Critical Quality Attribute
D_H	Average hydrodynamic diameter
DLS	Dynamic light scattering
EE	Encapsulation efficiency
EMA	European Medicines Agency
HPLC	High performance liquid chromatography
ICH	The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use
IN	Intranasal
IV	Intravenous
J	Flux
K_e	Elimination rate constant
K_p	Permeability coefficient
LC-MS/MS	Liquid chromatography with tandem mass spectrometry
MHLW	Ministry of Health, Labour and Welfare of Japan
MRT	Mean residence time
MWCO	Molecular weight cut-off
MX	Meloxicam
NaOH	Sodium hydroxide
P_{app}	Apparent permeability
PBS	Phosphate-buffered saline
PdI	Polydispersity index
PEG	Poly(ethylene glycol)
QbD	Quality by Design
QTPP	Quality Target Product Profile
SD	Standard deviation
SNES	Simulated Nasal Electrolyte Solution
SP	Soluplus®
$t_{1/2}$	Half-time

1. INTRODUCTION

The need for advanced drug delivery systems is based on the requirements of current patient expectations, the poor physicochemical properties of even the commonly applied active substances and the effective implementation of processability of the drug delivery systems via industrial techniques. This is especially true in case of nano drug delivery systems, such as polymeric micelles, and the pharmaceutical engineering techniques aiming to reach hardly accessible areas and target points in the human body like the central nervous system.

Regulatory expectations and limitations concern the nanomedical formulations as well. A structured and well-designed formulation study must be conducted accordingly to the currently effective guidelines. In lack of specific formulation study directing guidelines, which only state the subject of the investigation and not the manner of it, quality assurance techniques must be applied. In order to achieve the desired quality, safety and efficacy of the target product, the Quality by Design (QbD)-driven risk assessment can be a beneficial tool. The general nanoparticle characteristics of polymeric micelles, the administration route, the building co-polymer, the active substance and the required pharmacokinetic profile must be taken into consideration.

Thus, polymeric micelles loaded with small molecular weight active substances for brain targeting must be formulated in scope with this manner. Challenges regarding the intranasal, as an alternative drug delivery pathway can be tackled with the utilization of polymeric micelles. Since they can exert a solubilization effect up to an immense degree sided with permeability enhancement, rapid and burst-like drug release can be achieved. Compared to other carriers, polymeric micelles are also characterized with higher stability, especially the ones with poly(ethylene glycol) side chains. With proper stability and the advantageous effects, the active substance can be transported to the site of action, in our case, the central nervous system.

Polymeric micelles can be a solution as therapeutic carrier vehicles for numerous unmet clinical needs. A lot of potentially beneficial active substance also fail the clinical trials due to the incapability of administration to the target site. Thus, specific administration in many cases can be only solved with invasive techniques which does not necessary fulfil the required pharmacological effect. That is why the demand is stated based on current clinical and industrial feedbacks, which is the quality-controlled development of advanced drug delivery systems administered through the correct and therapeutically most efficient delivery route.

2. EXPERIMENTAL AIMS

This Ph.D. work aimed to prepare a novel, innovative meloxicam-loaded polymeric micellar formulation for intranasal administration under a quality-driven basis which can deliver the model drug to the central nervous system more efficiently compared to currently commercialized formulations. The fulfillment of the expected advanced pharmacokinetic profile lies in the proper colloidal and particle characteristics and the increased water solubility induced drug release and permeability enhancement. Thus, the research work was conducted accordingly to the following steps:

I. Based on our previous work, to establish a knowledge space and perform an initial risk assessment specified for the named active substance, applied micelle-forming co-polymer, the excipients, the formulation method and the desired drug delivery route. This was performed under the extended Quality by Design risk assessment procedure for the early stages of research and development process in a preclinical setting.

II. To optimize the formulation strategy and composition via factorial design to achieve nano particle sized, monodisperse polymeric micelles with adequate colloidal stability based on their zeta potential value. To determine the fulfillment of the intranasal administration: osmolality and viscosity.

III. To describe and evaluate the quantities and qualities related to the increase in water solubility. Also, to investigate the physical stability of the formulation in a long-term study accordingly to the International Council for Harmonization (ICH) Q1A guideline and to determine its physicochemical background.

IV. To describe the nasal applicability of the formulation based on the route of the carrier system. At first, *in vitro* drug release study was performed, followed by *in vitro* mucoadhesion study at nasal conditions. Followed by *in vitro* cytotoxicity and permeability studies on human RPMI 2650 nasal epithelial cell line, we aimed to describe the cellular effects and transport across the nasal mucosa. *Ex vivo* human, nasal mucosal permeability study was also performed. The polymeric micellar system meets the requirements if increased permeability values are achieved.

V. Lastly, we aimed to investigate the *in vivo* pharmacokinetic behavior of the formulation after nasal administration and to calculate and describe the related kinetic profile. To evaluate at this preclinical state the aimed burst-like and rapid drug transport to the central nervous system and whether our *in vitro* and *ex vivo* studies are validated.

3. MATERIALS AND METHODS

3.1. Materials

Meloxicam (MX) (4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide) was applied as model drug and acquired from EGIS Pharmaceuticals Plc. (Budapest, Hungary). Soluplus® (SP) (BASF GmbH, Hanover, Germany) was used as micelle-forming agent. 96 % *v/v* ethanol (Merck, Ltd., Budapest, Hungary) was used as organic solvent during our experiments. Microcrystalline sodium hydroxide (NaOH) as formulation excipient, chemicals for Simulated Nasal Electrolyte Solution (SNES) which combined 8.77 g sodium chloride (NaCl), 2.98 g potassium chloride (KCl), 0.59 g and anhydrous calcium chloride ($CaCl_2$) in 1000 ml of deionized water at pH 5.6 as well as disodium hydrogen phosphate (Na_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4) for pH 7.4 Phosphate-buffered saline (PBS) dissolution medium and the cryoprotectant D-trehalose dihydrate were acquired from Sigma-Aldrich Co., Ltd. (Budapest, Hungary). Purified water for the experiments was filtered using the Millipore Milli-Q® (Merck, Ltd., Budapest, Hungary) Gradient Water Purification System. All other chemicals were also obtained from Sigma-Aldrich Co., Ltd. if otherwise not indicated.

3.2. Quality by Design based risk assessment

As QbD is a knowledge- and risk assessment-focused approach, qualitative and quantitative risk factors, expressed as severity scores, must be presented. LeanQbD® Software (QbD Works LLC, Fremont, CA, USA) was used for the risk assessment procedure. At first, an interdependence rating amongst the QTPPs and the CQAs and amongst the CQAs and the CMAs/CPPs was performed. A three-level scale was used to describe the relation between these parameters: each relation was assigned with a "high" (H), "medium" (M) or "low" (L) attributive. The decision of assignment was performed based on numerous aspects including occurrence of the risk factor during formulation and/or final product development phase; controllability of the factor; whether the factor can be eliminated or can it be fixed at a certain value without affecting quality; detectability. The description of relations had the fundamental basis of how closely the factors are related and data from the collected literature, in addition to the regulatory aspects of a joint MHLW/EMA reflection paper on the development of block copolymer micelle medicinal products. Using the software, these qualitative relations were the basis of calculating the severity scores. The severity scores were transferred as percentages of the overall, cumulative severity scores and were depicted at the end of the interdependence tables.

3.3. Formulation of meloxicam-loaded polymeric micelles

First, a fixed value of 15 mg of MX was dissolved in 10 ml ethanol and 3 ml 1 M NaOH solution l under continuous stirring (750 rpm, 25 °C). The next step was to dissolve 100 mg of SP in the solution of MX. After 1 h of incubation, a Büchi R-210 (Büchi, Flawil, Switzerland) rotation vacuum evaporator was used to extract the solvent and a thin layer of matrix film was formed in the round-bottom flask. The temperature was set at 50 °C, with gradually decreasing pressure from 1000 to 100 mbar with a rate of 50 mbar/min, followed by 10 to 15 minutes of drying at 100 mbar. After hydration of the polymeric film with 6 ml of purified water, the pH was adjusted between 5.6 to 7.4 using diluted hydrochloride acid and 5 % w/v D-trehalose dihydrate was dissolved with the formulation. Thereafter, 1 ml aliquots of formulations were freeze-dried at – 40 °C for 12 h under a 0.013 mbar pressure with additional 3 h of secondary drying at 25 °C using a ScanVac CoolSafe 100-9 (LaboGene, ApS, Lynge, Denmark) laboratory apparatus. After the freeze-drying process, samples were reconstituted in 1 ml of purified water with a nominal MX concentration of 2.5 mg/ml. All measured materials were chosen based on the evaluated 3-factor, 3-level Box-Behnken factorial design discussed in the thesis.

3.4. Micelle characterization

3.4.1. Characterization of the optimized ex tempore dispersed formulation

The average hydrodynamic diameter (D_H) and the polydispersity index (PdI) were measured by the means of dynamic light scattering (DLS) using a Malvern Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK). The zeta potential of the formulations was also measured via this equipment. The formulations were dissolved in purified water, then measured at 25 °C in folded capillary cells with the refractive index of 1.720. Each measurement was carried out in triplicate with individual batches.

The reconstitution time of powder ampoules containing 2.5 mg/ml of MX was measured after adding 1.0 ml of purified water until a clean solution was formed under a constant stirring on a magnetic stirrer (100 rpm, 25 °C). After reconstitution, the pH of the colloidal solution was measured with WTW® inoLab® pH 7110 laboratory pH tester (Thermo Fisher Scientific, Budapest, Hungary). The osmolality of the in-water dispersed polymeric micelles was measured by means of an automatic osmometer (Knauer Semi-micro Osmometer, Berlin, Germany). Viscosity measurements were performed at 35 °C with a RheoStress 1 HAAKE instrument (Kalrsuhe, Germany) conducted with cone – plate geometry (radius: 49.9 mm, angle: 1°, gap: 0.052 mm). The apparent viscosity of the samples was measured over a shear

rate sweep of 0.01 – 100 s^{-1}. Each measurement was carried out in triplicate with individual batches.

3.4.2. Determination of thermodynamic solubility

The thermodynamic solubility of MX and the SP – MX formulation was determined in purified water (pH = 6.4) at 25 °C, in SNES (pH = 5.6) at 35 °C and PBS (pH 7.4) at 37 °C to present the pH and temperature conditions of the nasal mucosa and the blood vessels. 1 – 1 ml of the liquids were measured, and the products were dissolved until visible saturation. A 72-h incubation time followed the process at the predetermined temperatures with a set stirring speed of 100 rpm. After incubation, they were filtered through a 0.22 μm pore sized polyether sulfone membrane filter and the content of the dissolved drug was determined via HPLC. Each measurement was carried out in triplicate with individual batches.

3.4.3. Determination of encapsulation efficiency

To determine the encapsulation efficiency (EE) of the optimized formulation, the indirect method was applied. The MX – containing polymeric micelles were separated from the aqueous medium via centrifugation using a Hermle Z323 K high performance refrigerated centrifuge (Hermle AG, Gosheim, Germany) at 17,500 rpm at 4 °C for 45 min. The clear supernatant was diluted 10 – fold with purified water. Quantitative measurements of MX were performed via HPLC. Each measurement was carried out in triplicate with individual batches. The EE was calculated via the following the equation:

$$\mathrm{EE}(\%) = \frac{\mathrm{MX_{initial}\ (mg)} - \mathrm{MX_{measured}\ (mg)}}{\mathrm{MX_{initial}\ (mg)}} \cdot 100 \qquad (1)$$

3.4.4. Wetting properties and polarity calculation

OCA Contact Angle System (DataPhysics OCA 20, DataPhysics Inc., GmbH, Filderstadt, Germany) was used for studying the wettability of the optimized formulation in freeze – dried state. For the measurements, 0.10 g of powder was compressed under a pressure of 1 t by a Specac® hydraulic press (Specac Inc., Fort Washington, PA, USA). The liquid media used for the contact angle measurements included bidistilled water (interfacial tension of polar component (γ_i^p) = 50.2 mN/m, interfacial tension of disperse component (γ_i^d) = 22.6 mN/m) and diiodomethane (γ_i^p = 1.8 mN/m, γ_i^d = 49 mN/m). The contact angles of pressings were determined applying the method of Wu. The solid surface free energy is the sum of the polar (γ_i^p) and nonpolar (γ_i^d) components and was calculated according to the Wu equation (Eq. 2.):

$$(1 + \cos\Theta)\gamma_l = \frac{4(\gamma_s^d \gamma_l^d)}{\gamma_s^d \gamma_l^d} + \frac{4(\gamma_s^p \gamma_l^p)}{\gamma_s^p \gamma_l^p} \quad (2)$$

where Θ is the contact angle, γ_s is the solid surface free energy and γ_l is the liquid surface tension.

The percentage polarity can be calculated from the γ^p and γ values (Eq. 3):

$$\text{Percentage polarity } (\%) = \frac{\gamma^p}{\gamma} \cdot 100 \quad (3)$$

3.4.5. Raman spectroscopic measurement

For the Raman spectroscopic measurement of polymeric micelles, a Thermo Fisher DXR Dispersive Raman Instrument (Thermo Fisher Scientific, Inc., Waltham, MA, USA) equipped with a charge-coupled device (CCD) camera and a diode laser operating at 780 nm was used. Raman measurements were carried out with a laser power of 12 mW at 25 μm slit aperture size. The set exposure time was 2 s and the acquisition time was 6 s, for a total of 32 scans per spectrum in the spectral range of 3500 – 200 cm^{-1} with cosmic ray and fluorescence corrections. The Raman spectra were normalized in order to eliminate the intensity deviation between the measured areas.

3.5. Long-term stability study

To determine the physical stability after freeze – drying the product in solid state, a long – term stability study was conducted in accordance with the ICH Q1A guideline in a refrigerator for 12 months at 5 ± 3 °C. Each month a portion was dissolved in purified water and then D_H and PdI were measured via the described DLS method. Each measurement was carried out in triplicate with individual batches.

3.6. Nasal applicability studies

3.6.1. In vitro drug release study

The modified paddle method (Hanson SR8 Plus (Teledyne Hanson Research, Chatsworth, CA, USA) was used to examine the rate of drug release of the SP-MX formulation compared to raw MX (suspended via 0.1% w/v hyaluronic acid solution). 1 – 1 ml of the dissolved SP-MX formulation and the MX suspension were placed in dialysis bags (Spectra/Por® Dialysis Membrane with a MWCO value of 12-14 kDa (Spectrum Laboratories Inc., Rancho Dominguez, CA, USA)). 100 ml of SNES was applied as a dissolution medium at 35 °C. Each measurement was carried out in triplicate with individual batches.

3.6.2. In vitro cell viability and permeability study on human RPMI 2650 cell line

For the *in vitro* cell line studies, human nasal epithelial cells (RPMI 2650; ATCC cat. no. CCL 30) were applied. For the permeability measurements, epithelial cells were co-cultured with human vascular endothelial cells to create a more physiological barrier representing both the nasal epithelium and the submucosal vascular endothelium. To follow cell damage and/or protection in living barrier forming cells and to quantify the viability of adherent cells, real-time cell electronic sensing technique was used. To register the impedance of cell layers, a RTCA-SP instrument (ACEA Biosciences, USA) was used. The registration took place every 10 min, and the cell index was defined at each time point. The cell index was defined as $(R_n - R_b)/15$, where R_n is the cell-electrode impedance of the wall when it contains cells and R_b is the background impedance. E-plates with 96-well and built-in gold electrodes were coated in 0.2% gelatin and placed for 20 min in the incubator. After the incubation, the gelatin was removed and 50 µl of the culture medium was added to each well. The dispensed RPMI 2650 cell suspension had a density of 2×10^4 cells/well. When cells reached a steady growth phase, they were treated with SP-MX and the building components. For the cell viability measurements 10×, 30× and 100× dilutions from SP-MX formulation as well as 2.5 mg/ml MX suspension, 83.3 mg/ml D-trehalose dihydrate, 16.7 mg/ml SP solutions, corresponding to the concentration of components in the SP-MX formulation were prepared in cell culture medium.

For the permeability measurements, RPMI 2650 cells were cultured on inserts (Transwell, polycarbonate membrane, 3 µm pore size, 1.12 cm^2, Corning Costar Co., MA, USA) placed in 12-well plates in the presence of endothelial cells for 5 days. To prepare the co-culture model, endothelial cells were passaged (1×10^5 cells/cm^2) to the bottom side of tissue culture inserts coated with low growth factor containing Matrigel (BD Biosciences, NJ, USA) and nasal epithelial cells were seeded (2×10^5 cells/cm^2) to the upper side of the membranes which were coated with rat tail collagen. For the permeability experiments the inserts were transferred to 12-well plates containing 1.5 ml Ringer-HEPES buffer in the acceptor compartments. In the donor compartments 0.5 ml buffer was pipetted containing MX or the SP-MX polymeric micelle formulation. The plates were kept on a horizontal shaker (120 rpm) during the assay which lasted for 60 min. Samples from both compartments were collected and the MX concentration was measured by HPLC The cleared volume was calculated from the concentration difference of the tracer in the acceptor compartment ($\Delta[C]_A$) after 30 min and in donor compartments at 0 hour ($[C]_D$), the volume of the acceptor compartment (V_A; 1.5 ml) and the surface area available for permeability (A; 1.1 cm^2) using Eq. 4.:

$$P_{app}\left(\frac{cm}{s}\right) = \frac{\Delta[C]_A x\, V_A}{A\ x\ [C]^D x \Delta t} \quad (4)$$

For the permeability measurement, 2.5 mg/ml MX and 10× dilution of SP-MX were prepared in Ringer-Hepes buffer and added to the donor compartments.

3.6.3. Ex vivo nasal permeability study

To investigate *ex vivo* the transmucosal passive diffusion, a modified Side-Bi-Side® type horizontal diffusion apparatus was applied. The diffusion of MX suspension (2.5 mg/ml) and SP-MX formulation was tested across the excised human nasal mucosa. The experiments have been carried out under approval of University of Szeged's institutional ethics committee (ETT-TUKEB: IV/3880-1/2021/EKU). The nasal mucosa was mounted between the donor and the acceptor compartments with a diffusion surface area of 0.785 cm^2. The donor was prepared by mixing 1.0 ml of the formulations with 8.0 ml of SNES while the acceptor phase contained 9.0 ml of pH 7.4 PBS to simulate nasal conditions. The temperature of the chambers was controlled at 35 ± 0.5 °C using a heating circulator (ThermoHaake C 10-P5, Sigma-Aldrich Co., Ltd., Budapest, Hungary). The compartments were continuously stirred at 100 rpm using magnetic stirrers. 100 µl aliquots from the acceptor phase were taken at predetermined time points and immediately replaced with fresh medium. The concentration of MX was determined by HPLC. The flux (J (µg/cm^2/h)) was calculated from the permeated drug quantity through the nasal mucosa (m_t), divided by the surface of the membrane insert (A) and the duration of the investigation (t) (Eq. 5). The permeability coefficient (K_p (cm/h)) was determined by dividing the flux (J) with the drug concentration in the donor phase (C_d (µg/cm^3)) as seen in Eq. 6.

$$J = \frac{m_t}{A\ x\ t} \quad (5)$$

$$K_p = \frac{J}{C_d} \quad (6)$$

3.6.4. In vivo pharmacokinetic study

All the experiments involving animal subjects were carried out with the approval of the National Scientific Ethical Committee on Animal Experimentation (permission number: IV/1247/2017). The animals were treated in accordance with the European Communities Council Directives (2010/63/EU) and the Hungarian Act for the Protection of Animals in Research (Article 32 of Act XXVIII). A 60-µg dose of MX from the 2.5 mg/ml SP-MX formulation was administered into the right nostril of 160 – 180 g male Sprague Dawley rats (*n*=24) via a pipette. At predetermined time points (5, 15, 30, 60, 120 and 240 min), the blood

of the rats (under isoflurane anaesthesia) was collected into heparinized tubes via cardiac puncture. Then, the animals were sacrificed by decapitation and brain tissues were removed, rinsed in ice-cold PBS, weighed and stored at – 80 °C until assayed.

Pharmacokinetic parameters were analysed via PK Solver 2.0 Software through non-compartmental analysis of the measured brain and plasma data. The area under the curve (AUC) of the time (min) – concentration curves of each animal were fitted with a linear trapezoidal method. All reported data are means ± SD (n = 4).

3.7. Quantitative analysis of meloxicam

To quantify the concentration of MX during our measurements, high performance liquid chromatography was applied (Agilent 1260 (Agilent Technologies, Santa Clara, USA)). The stationary phase was a Kinetex® EVO C18 column (5 μm, 150 mm x 4.6 mm (Phenomenex, Torrance, CA, USA)). A two-step gradient elution was used for the separation using 0.065 M KH_2PO_4 solution (pH 2,8) and methanol as eluents. The detection of the chromatograms was carried out at 355 ± 4 nm using UV-Vis diode array detector. Data were evaluated using a ChemStation B.04.03 software (Agilent Technologies, Santa Clara, CA, USA).

The quantitative analysis of MX for the *in vivo* studies was performed by using a Waters Acquity I-Class UPLC™ system (Waters, Manchester, UK), connected to a Q Exactive™ Plus Orbitrap mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) equipped with a heated ESI ion source. As internal standard piroxicam was used. Gradient chromatographic separation was performed at room temperature on a Acquity BEH C18 column (50 mm × 2.1 mm, particle size 1,7 μm) protected by a C18 guard column (2 × 2 mm, Phenomenex, Torrance, CA, USA) by using 0.1% aqueous formic acid as Solvent A and acetonitrile with 0.1% formic acid as Solvent B. The mass spectrometer was used in a positive mode with the following parameters of the heated electrospray ionization source: spray voltage at 3.5 kV, capillary temperature at 256 °C, aux gas heater temperature at 406 °C, sheath gas flow rate at 47.5 l/h, aux gas flow rate at 11.25 l/h, sweep gas flow rate at 2.25 l/h, and S-lens radio frequency level at 50.0 (source auto-defaults). Parallel-reaction-monitoring mode was used for quantifying by monitoring the following transitions: m/z 352 → 115 for MX and m/z 332 → 95 for piroxicam. Data acquisition and processing were carried out using Xcalibur and Quan Browser (version 4.5.474.0) software (Thermo Fisher Scientific, San Jose, CA, USA).

4. RESULTS AND DISCUSSION

4.1. Quality by Design based risk assessment

As part of a QbD-based formulation study, a proper knowledge space is wished to be set up followed by the critical analysis of the collected variables by initial risk assessment and later by the evaluation of a factorial design. During our research work, previously we reported a general knowledge space development on polymeric micelles in general where numerous articles were analyzed to find all the variables affecting the safety, efficacy and quality of this specific nano drug delivery system. Based on our findings, in addition to the EMA regulations, specific QTPPs, CQAs, CMAs and CPPs were identified. After the identification, the next step was to perform the risk assessment amongst the determined CQAs and QTPPs (Figure 1.)

QTPPs / CQAs	Indication	Target patients	Administration route	Site of activity	Absorption feature	Drug release profile	Nanocarrier	Severity score (%)
Particle size	MEDIUM	LOW	HIGH	HIGH	HIGH	HIGH	HIGH	21.84
Excipients	LOW	LOW	MEDIUM	MEDIUM	HIGH	HIGH	HIGH	17.51
Solubility	MEDIUM	LOW	HIGH	MEDIUM	HIGH	HIGH	HIGH	11.49
Drug release time	LOW	LOW	HIGH	MEDIUM	MEDIUM	HIGH	HIGH	8.92
Permeability rate	HIGH	LOW	HIGH	HIGH	HIGH	MEDIUM	MEDIUM	8.49
Encapsulation efficiency	MEDIUM	LOW	MEDIUM	MEDIUM	HIGH	HIGH	MEDIUM	8.03
Wettability	MEDIUM	LOW	MEDIUM	MEDIUM	HIGH	HIGH	MEDIUM	6.26
Structure	LOW	LOW	MEDIUM	LOW	MEDIUM	MEDIUM	MEDIUM	5.75
Physical appearance	LOW	LOW	MEDIUM	HIGH	MEDIUM	MEDIUM	LOW	5.06
Stability	LOW	LOW	LOW	LOW	MEDIUM	LOW	LOW	3.77
Toxicity	LOW	LOW	MEDIUM	LOW	LOW	LOW	LOW	2.88

Figure 1. Interdependence rating amongst the CQA and QTPP elements. All connections are described with high/medium/low relations. The last column represents the severity score percentage of the CQA elements in decreasing order compared to the cumulative severity scores.

The first interdependence rating revealed that particle characteristics are much more risky factors compared to the nasal administration related factors. This theorem is highly supported as most factorial design-based optimization processes would consider these factors as the main dependent variables. Our quality criteria are that the particle size should be between 10 to 200 nm in monodisperse distribution. The excipients, such as the SP itself was predetermined, therefore even though the risk is high, it has a set qualitative aspect. However quantitatively it should be optimized. The following CQAs on the list of the first column were all later-on

investigated factors. The next step was to determine which CMA/CPP factors can be characterized with high-risk ratios, therefore an interdependence rating amongst CQAs and CMAs/CPPs was evaluated (Figure 2.)

CMA/CPPs / CQAs	Composition	Mixing time	Rotation pressure	Rotation temperature	Rotation speed
Particle size	HIGH	HIGH	MEDIUM	MEDIUM	MEDIUM
Excipients	HIGH	MEDIUM	LOW	MEDIUM	LOW
Solubility	HIGH	HIGH	HIGH	MEDIUM	MEDIUM
Drug release time	HIGH	MEDIUM	LOW	LOW	MEDIUM
Permeability rate	HIGH	MEDIUM	MEDIUM	MEDIUM	MEDIUM
Encapsulation efficiency	HIGH	HIGH	MEDIUM	MEDIUM	LOW
Wettability	HIGH	MEDIUM	MEDIUM	MEDIUM	LOW
Structure	HIGH	LOW	LOW	MEDIUM	LOW
Physical appearance	MEDIUM	MEDIUM	LOW	LOW	MEDIUM
Stability	HIGH	LOW	LOW	LOW	LOW
Toxicity	MEDIUM	MEDIUM	LOW	LOW	LOW
Severity score (%)	47.78	21.88	15.39	7.82	7.13

Figure 2. Interdependence rating amongst the CQA and CMA/CPP elements. All connections are described with high/medium/low relations. The last row represents the severity score percentage of the CMA/CPP elements in decreasing order compared to the cumulative severity scores (%).

Based on the risk assessment specified on the thin-film hydration method aiming to formulate optimized polymeric micelles, it has been revealed that the composition of the formula has the highest risk factor besides time. Therefore, later the composition of each excipient was chosen as part of the factorial design. MX concentration was set with the goal of 2.5 mg/ml in the final product. The mixing time was not set prior the optimization process as volumes would vary prior to solvent extraction. The criterion was that it should be visibly dry after appropriate extraction.

4.2. Micelle characterization

4.2.1. Characterization of the ex tempore dispersed formulation

After the optimization via Box-Behnken design, the characteristic of the in-water dispersed nasal formulation was the following. The measured average hydrodynamic diameter (D_H) value was 111.6 ± 3.0 nm, which is optimal and in the typical particle size range of polymeric micelles (10 to 200 nm). This particle size reduction can be categorized in the nanoencapsulation techniques, where the reduction is achieved via additional excipients, such as the co-polymer

SP applied in our study. Not only it would hold the benefits of a nanoparticle with increased solubility and surface area, but the building co-polymer's beneficial properties would also come to surface. As SP micelles have poly(ethylene glycol) (PEG) as their hydrophilic corona, it would also increase the stability in the circulation and during biodistribution. It would also increase the residence time in the blood without degradation and prolong the efficient time during therapy. Besides micelle size, its distribution is of paramount importance as well. PdI refers to the micelle size distribution, which in the case of the SP-MX formulation was 0.114 ± 0.06. PdI values below 0.3 are considered monodisperse, therefore our formulation met this criterion. Not only does it affect the colloidal structure and properties of the nanocarrier, but it would also affect the drug release and permeation profile. Uniform drug release and permeation would only be possible if the formulation is monodisperse, as highly variable and polydisperse formulation would cause fluctuations in these kinetic profiles. Zeta potential refers to the surface charge properties of the colloidal system is liquid state. In case of SP-MX formulation, the measured zeta potential was – 25.2 ± 0.4 mV. It is a relatively high absolute value, which prevents the aggregation of the dispersed nanoparticles due to the repulsion forces amongst them. Polymeric micelles with negative surface charge or without charge can also be describes as mucopenetrating micelles, where the mucus layer allows the micellar carrier to be absorbed via transcytosis or paracellular transport.

The reconstitution time of the freeze-dried SP-MX formulation was 2 s, which far exceeds the requirements of the European Pharmacopoeia (Ph. Eur. 10), stating that a freeze-dried product should be constituted to a solution using an appropriate diluent prior to patient administration in less than 3 min. The physiological pH in the nose is between 5.6 to 7.4 which allows normal ciliary function. Also in this slightly acidic environment, lysozyme, which is a natural antimicrobial agent in the nasal cavity, can exert its beneficial effects, therefore it is advised not to change this. Besides this, irritation could occur if major deviations would take place. The SP-MX formulation had a pH of 6.49 which fits this criterion. Nasal preparations in general are normally isotonic (about 290 mOsmol/l), however deviation from isotonicity can also be beneficial. Hypertonic solutions can cause a shrinkage in the epithelial cell layer and can inhibit the normal ciliary activity. In our case, a hypotonic solution (240 ± 12 mOsmol/l) was obtained, which can increase drug absorption. The viscosity of the formulation was 32.5 ± 0.28 mPas which is sufficiently low enough that can be administered via a nasal spray device. Also, it helps with the faster drug release, as the matrix area for diffusion is not concise to the extent where it would prolong the drug liberation from the carrier.

4.2.2. Determination of thermodynamic solubility and encapsulation efficiency

The thermodynamic solubility of the SP-MX formulation compared to initial MX was measured via the saturation method at 3 different pH and temperature conditions representing the nasal, storage and the blood conditions. At 5.6 pH and 35 °C represents the nasal conditions, the 6.4 pH at 25 °C the storage conditions after *ex tempore* dispersion and the 7.4 pH at 37 °C the blood conditions. The calculated solubility – related factors can also be found in Table 1. besides the measured encapsulation efficiency by the indirect method. The solubility of the SP-MX formulation is significantly higher in all cases which can be contributed with the nano particle size in monodisperse distribution and the high (approx. 90%) encapsulation efficiency.

Table 1. Solubility and the encapsulation efficiency of the SP-MX formulation (S_{tot}) compared to raw MX (S_w). Data are presented as means ± SD from 3 individual batches.

pH	S_w (mg/l)	S_{tot} (mg/l)	EE (%)
5.6	6.92 ± 0.19	5419.7 ± 1.284	89.4 ± 2.7
6.4	6.99 ± 0.45	5527.0 ± 1.197	92.1 ± 1.5
7.4	7.09 ± 0.33	5775.9 ± 0.789	89.9± 3.6

4.2.3. Wetting properties and polarity calculation

Wetting properties are of paramount importance if the aim is to develop a product with rapid reconstitution in water. The contact angle of MX against water is high showing high hydrophobicity, whilst the SP polymeric micelle-forming co-polymer and the SP-MX formulation had relatively low contact angles (Table 2.). The calculated polarity values also prove that an increase of polarity is experienced after the solubilization of MX with the nanocarrier. The polarity increase can be due to the high volume and mass ratio of the polar D-trehalose dihydrate in the formulation and the high polarity of SP itself. The high polarity alongside the high encapsulation efficiency can explain the short reconstitution time of approx. 2 s and the increased thermodynamic water solubility as measured before.

Table 2. Contact angles, the calculated surface free energies and the polarity values of the SP-MX formulation (in freeze-dried state) compared to the initial MX and SP co-polymer. Data of contact angles are presented as means ± SD (*n*=3 independent formulations).

Samples	Θ_{water} [°]	$\Theta_{diiodomethane}$ [°]	γ^d [mN m^{-1}]	γ^p [mN m^{-1}]	γ [mN m^{-1}]	Polarity (%)
MX	75.3±5.2	16.2±3.3	44.7	9.3	54.0	17.1
SP	33.4±0.3	16.4±0.0	44.02	29.20	73.22	39.6
SP-MX	11.3±0.5	23.4±0.1	42.08	37.13	79.21	47.0

4.2.4. Raman spectroscopic measurement

Raman spectra of initial MX, SP, the physical mixture and the SP-MX formulation is shown in Figure 3. Aromatic ring vibration of benzothiazine at 1598 cm^{-1} can be found in the spectrum of MX, which derives from the bending vibration of CH_2 and the stretching vibration of C=C or C=N groups. A sharp peak can be also found at 1532 cm^{-1} which indicates a stretching vibration of C=N in the thiazole ring of the molecule. Some other characteristics can be found at the following peaks: 1305 cm^{-1} (ν_{OH}), 1267 cm^{-1} ($\nu_{C\text{-}N\text{-}C}$) and 1164 cm^{-1} ($\nu_{C\text{-}S}$). A shift can be found in the SP-MX formulation as the band located at 1305 cm^{-1} in case of raw MX has shifted to higher energy at 1394 cm^{-1} as a result of deprotonation. The appearance of a new peak due to the deprotonation of phenolic OH changed the vibrational motion of C=C and C–N bonds. The magnitude of 89 cm^{-1} reflects the OH group attached to the 6-membered heteroatom ring attends in the hydrogen bond formation with SP.

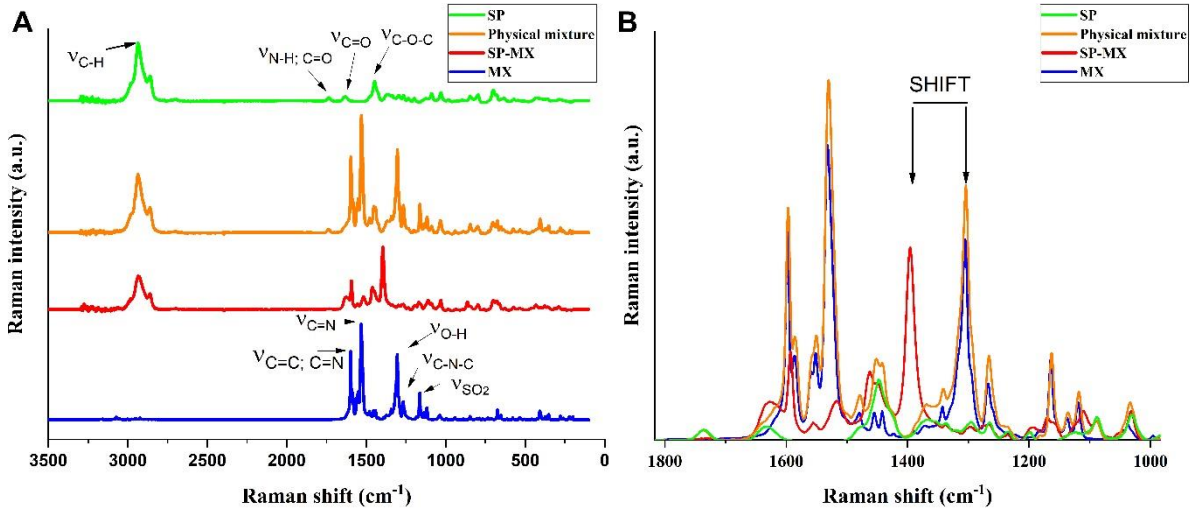

Figure 3. (A) Raman spectra of MX, SP, the physical mixture and the SP-MX formulation and (B) the enlarged region of the structural changes

4.3. Long-term physical stability study

In order to investigate the physical stability in solid state, a long-term stability study was conducted for 12 months. As the main goal was to formulate an *ex tempore* dispersible nasal product, each month a portion of the freeze-dried powder was dissolved in purified water followed by dynamic light scattering measurements. The DH and the PdI values were measured as main critical parameters. To calculate the absolute change, data value of the measurement of the freshly prepared formulation was subtracted from the final, 12th month measurement point. The DH increased by 3.31 nm and the PdI by 0.016 after 12 months of storage (Table 6.). The results also prove that 5 % w/w D-trehalose dihydrate was a suitable chose as a cryoprotectant.

4.4. Nasal applicability studies

4.4.1. In vitro drug release study

In vitro drug release study was performed under simulated nasal conditions. The higher drug release of the SP-MX formulation can be derived to the increased solubilized concentration which equals to the thermodynamic water solubility of MX in the formulation compared to raw MX alongside with the high polarity and nano particle size range. In the first 15 to 20 minutes, a burst effect can be experienced which is typical for drug delivery systems with rapid drug release (Figure 4.). It is critical in nasal administration, as the average residence time is approximately less than 20 minutes. Therefore, the SP-MX formulation met this criterion.

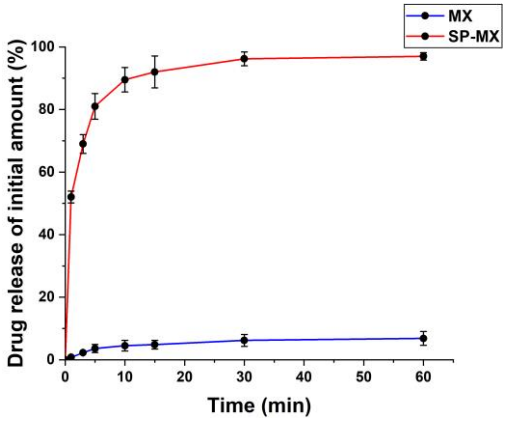

Figure 4. *In vitro* drug release curve of the SP-MX nanoformulation compared to initial MX suspension, measured via the dialysis bag method. All data are presented as means ± SD from 3 individual batches.

4.4.2. In vitro cell viability and permeability study on human RPMI 2650 cell line

The cell viability assay proved that all components of the SP-MX formulation and SP-MX itself is non-toxic for the nasal cell line. This is corroborated with the high safety profile of SP, as clinical trials stated that 114 mg/kg of SP can be considered safe. Figure 5. shows the normalized cell ratio compared to the positive control physiological saline solution and the negative control Triton-X 100 detergent. This also proves the superiority of polymeric micelle forming co-polymers compared to classic surfactants, as they are less likely to cause nasal irritation and toxicity.

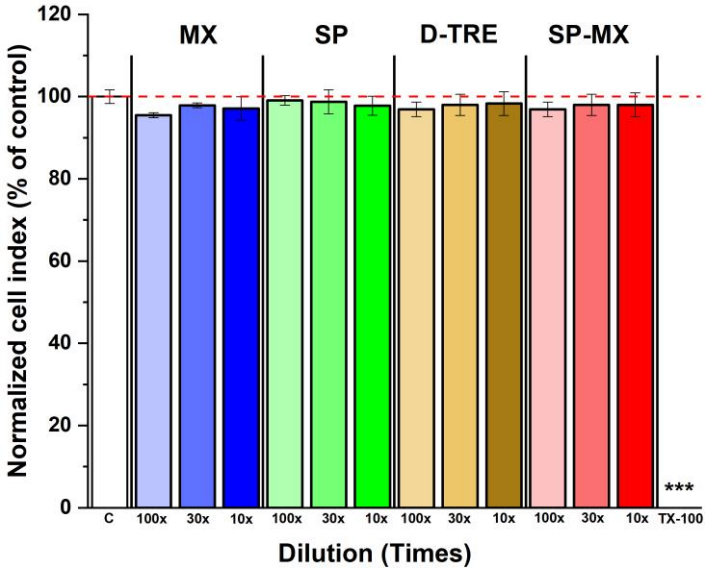

Figure 5. Cell viability of RPMI 2650 nasal epithelial cells after the treatment of SP-MX and its components measured by impedance. The values are presented as a percentage of the control group and as means ± SD (*n*= 6-12). Statistical analysis: ANOVA followed by Dunett's test. ***$p < 0.01$.

MX permeability across the nasal co-culture model showed a 5-fold increase compared to raw MX after the 1-hour treatment. The P_{app} value for MX was 8.265×10^{-6} cm/s and for the SP-MX formulation, it was 5.1116×10^{-5} cm/s. This significantly higher (***, $p < 0.001$) apparent permeability values can be validated by the nano particle size and the permeability enhancing effect of SP besides the increase in water solubility*Ex vivo nasal permeability study*

Ex vivo permeability measurements were carried out in order to describe the passive diffusion tendency of the SP-MX formulation compared to the initial MX. After quantification of the aliquots via HPLC, the cumulative permeability was calculated and was depicted as a function of time as it can be seen in Figure 6.

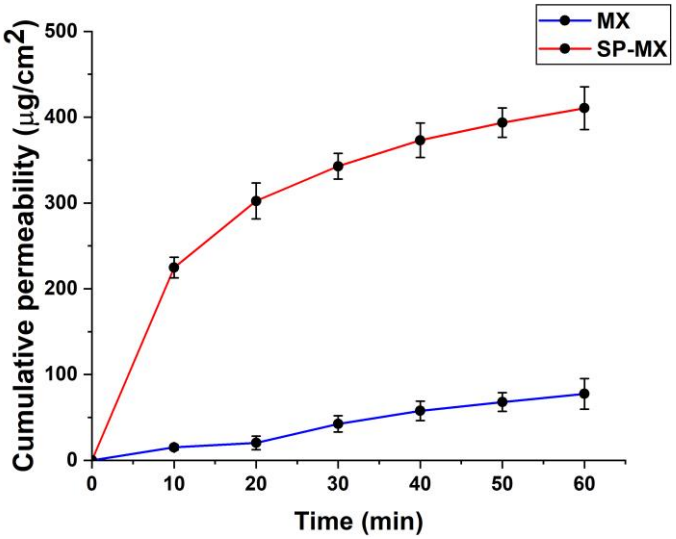

Figure 6. Result of the *ex vivo* quantitative permeability measurement across human nasal mucosa performed in a horizontal Side-bi-Side®-type diffusion cell. The cumulative permeability of SP-MX compared to MX is shown as a function time. All measurement were carried out in triplicate and data are presented as means ± SD (*n*= 3).

The higher cumulative permeability values are contributed to the rapid and high-degree drug release of the formulation compared to raw MX. The nano size range also favors drug penetration across mucosal barriers. A burst-like drug permeation can be found in the first 15 to 20 minutes of the measurement similarly to the drug release study. This also proves that the moderate mucoadhesion would not affect this desired aim. Since the burst-like drug release and permeation are both presented in the same amount of time, no lag time could be expected resulting in immediate absorption. The calculated permeability coefficient is 0.493 cm/h and the calculated flux at the final time point is 410.54 μg/cm^2 regarding the SP-MX formulation. These values are significantly higher compared to the reference initial MX, where they were 0.068 cm/h and 77.491 μg/cm^2, respectively (** $p < 0.001$ in both cases).

4.4.3. In vivo pharmacokinetic study

During the *in vivo* pharmacokinetic study with the intranasally administered SP-MX formulation, plasma and brain concentration levels were determined via the described LC-MS/MS analytical method. The plasma and brain concentrations can be found on Figure 7.

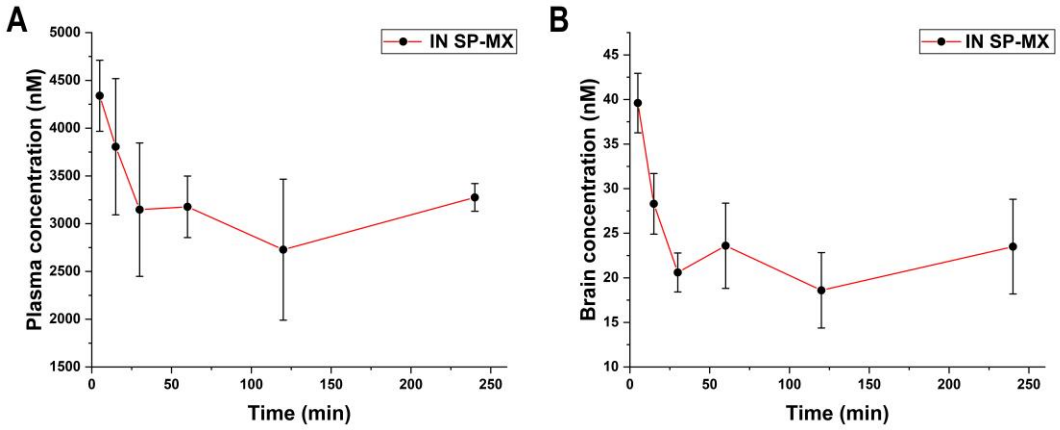

Figure 7. Results of the *in vivo* measurements in Sprague Dawley rats after the intranasal administration of the SP-MX formulation. (A) Plasma concentration levels and (B) Brain concentration levels of MX. Data are presented as means ± SD (n = 4).

As a reference data, our research group's intranasal and intravenous (IV) reference data were used in this discussion section. Plasma levels did not reach the level of the IV data, which is reasonable as it is a natural law that the entire API enters the bloodstream immediately after IV administration. However, the brain concentrations exceed our previous findings. Bartos et al. investigated the effect of size reduction techniques such as spray drying on MX and its brain targetability after intranasal (IN) administration. In their case, the achieved MX concentration was less than 1 nM in all cases: IN and IV administration. Compared to our results, approx. 35 to 40-times higher concentration were achieved regarding the brain levels. The higher AUC values also indicate that higher effective MX concentration was achieved in the central nervous system. See: approx. 35 nM × min in case of the spray-dried formulation vs. approx. 5000 nM × min in case of the SP-MX formulation. The superiority of nanocarriers over classic, only physical particle size reduction techniques have been proven. As mentioned earlier, *in vitro* and *ex vivo* studies corroborated that a burst-like drug release and absorption could be expected after IN administration. The highest concentration in the brain appeared after 5 minutes, which fact corroborates this theorem. A second new peak can be observed at lower concentrations at the 60-minute time interval, which indicates that a slight portion of MX reached the brain after the delay. The rapid onset of action predicts the nose-to-brain rapid and direct transport, whilst the later one describes a typical nose-to-blood carrier system prior to blood-brain barrier (BBB) penetration. A slight increase can be observed at the final time point, meaning that prolonged effect and central nervous system targeting could be also achieved. The supporting pharmacokinetic parameters can be found in Table 3. calculated from the plasma and brain concentration vs. time curves.

Table 3. Calculated pharmacokinetic parameters of the IN administered SP-MX formulation. All data are presented as means ± SD (n = 4). Abbreviations: K_e – elimination rate constant; $t_{1/2}$ – half-time; AUC_{0-t} – area under curve value between 0 and the measurement time (t); $AUC_{0-\infty}$ -area under curve value between 0 and infinity; Cl – Clearance; MRT – mean residence time.

Pharmacokinetic parameter	Plasma	Brain
K_e (min^{-1})	$0.00091 \pm 2.2 \cdot 10^{-4}$	$0.00132 \pm 3.6 \cdot 10^{-5}$
$t_{1/2}$ (h)	804.143 ± 22.362	525.174 ± 14.378
AUC_{0-t} (µmol/ml × min)	811.228 ± 12.281	5.263 ± 0.051
$AUC_{0-\infty}$ (µmol/ml × min)	5115.035 ± 124.78	22.991 ± 0.674
Cl (µg/kg)/(nmol/ml)/min	$7.6 \cdot 10^{-5} \pm 1.6 \cdot 10^{-5}$	$0.0163 \pm 4.8 \cdot 10^{-4}$
MRT (min)	1197.25 ± 315.44	796.337 ± 23.17

Based on the calculations, the AUC values of the brain are significantly higher to the previously mentioned spray-dried formulations where only physical particle size-reduced MX was presented in the system. The clearance and MRT values of the formulation indicates a slow elimination progress. This can be explained the residence time prolonging effect of PEG 6000, which is the outer shell of the polymeric micelle formulation. As the plasma curves entered to the natural elimination pathway, the early high concentration of MX in the brain can be further supported by the possibility of nose-to-brain transport. As mentioned earlier, regarding the zeta potential measurement, the SP-MX formulation has a negative surface charge which indicates a mucopenetrating effect across the nasal mucosa allowing carrier-intact direct axonal transport and rapid absorption to the blood. This is supported by the *in vitro* cell line and *ex vivo* permeability studies as well, since tremendously higher concentration of MX was achieved.

5. CONCLUSION

Since the poor physicochemical properties of commonly applied active substances are limiting the effective therapy of neurodegenerative diseases, polymeric micelles as a smart carrier system can tackle these challenges as well as improve the patient adherence to therapy through intranasal delivery. In accordance with our research goals, a novel MX-loaded polymeric micelle formulation was designed on a QbD basis aimed for nasal administration as a potentially most efficient pathway to reach the central nervous system. The main conclusions can be summarized in the following points:

I. We successfully determined the critical factors affecting the product quality, safety and efficacy. A two-step risk assessment was evaluated to prove this, based on interdependence and probability rating.

II. After the evaluation of the design space based on the results of the factorial design, the achieved polymeric micelles had adequate particle size of 111.6 nm in monodisperse size distribution and proper colloidal stability with a zeta potential value of – 25.2 mV. The SP-MX formulated fitted the criteria of *ex tempore* dispersible freeze-dried powders. The pH was adjusted to fit the nasal pH range. The viscosity of the formulation makes it applicable through a nasal spray nozzle and the hypotonic nature of the formulation allows rapid drug absorption.

III. A high encapsulation efficiency of approx. 90 % was achieved which contributed to the immense increase in water solubility compared to raw MX. The physical stability of the formulation is satisfying due to the formation of hydrogen bonds between MX and the micelle-forming co-polymer, SP.

IV. *In vitro* drug release study proved that a rapid, burst-like effect can be achieved with the SP-MX formulation. *In vitro* cell line studies showed safe administration of the formulation through nasal administration alongside with higher permeation across the cells. The later was also corroborated with *ex vivo* nasal mucosal permeation study where the increased flux value refers to the permeability enhancement.

V. As tested at *in vivo* conditions, the main goal was achieved as MX has been promoted to the central nervous system via nasal administration in a higher degree than intravenous reference solutions and particle size reduced MX formulations measured by our research group before. Thus, the superiority of nanoencapsulation techniques, such as polymeric micelle formation has been proved worthy over the physical particle size reducing techniques.

PUBLICATIONS RELATED TO THE THESIS

I. **Sipos, B.**, Szabó – Révész, P., Csóka, I., Pallagi, E., Dobó, D. G., Bélteky, P., Kónya, Z., Deák, Á., Janovák, L., Katona, G. (2020). Quality by Design based formulation study of meloxicam-loaded polymeric micelles for intranasal administration. *Pharmaceutics*, 12(8), 697. **(Q1, IF: 6.321, Citation: 30)**

II. **Sipos, B.**, Katona, G., Csóka, I. (2021). A systematic, knowledge space-based proposal on quality by design-driven polymeric micelle development. *Pharmaceutics*, 13(5), 702. **(Q1, IF: 6.525, Citation: 8)**

III. **Sipos, B.**, Katona, G. (2022). Innovatív polimer alapú nanohordozók a központi idegrendszer betegségeinek kezelésére. *Gyógyszerészet*, 66, 182 – 188.

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I. Katona, G., **Sipos, B.**, Budai – Szűcs, M., Balogh, G.T., Veszelka, S., Gróf, I., Deli, M.A., Volk, B., Szabó – Révész, P., Csóka, I. (2021). Development of in situ gelling meloxicam – human serum albumin nanoparticle formulation for nose – to – brain application. *Pharmaceutics*, 13(5), 646. **(Q1, IF: 6.525, Citation: 10)**

II. Sabir, F., Katona, G., Ismail R., **Sipos, B.**, Ambrus, R., Csóka, I. (2021). Development and characterization of N-propyl gallate encapsulated solid lipid nanoparticles – loaded hydrogel for intranasal delivery. *Pharmaceuticals*, 14(7), 696. **(Q1, IF: 5.215, Citation: 7)**

III. **Sipos, B.**, Csóka, I., Budai – Szűcs, M., Kozma, G., Berkesi, D., Kónya, Z., Balogh, G.T., Katona, G. (2021). Development of dexamethasone – loaded mixed polymeric micelles for nasal delivery. *European Journal of Pharmaceutical Sciences*, 166, 105960. **(Q1, IF: 5.112, Citation: 7)**

IV. Katona, G., **Sipos, B.**, Ambrus, R., Csóka, I., Szabó – Révész, P. (2022). Characterizing the drug – release enhancement effect of surfactants on megestrol-acetate – loaded granules. *Pharmaceuticals*, 15(2), 113. **(Q1, IF: 5.215, Citation: 2)**

V. Dobó, D.G., Németh, Z., **Sipos, B.**, Cseh, M., Pallagi, E., Berkesi, D., Kozma, G., Kónya, Z., Csóka, I. (2022). Pharmaceutical development and design of thermosensitive liposomes based on the QbD approach. *Molecules*, 27(5), 1536. **(Q1, IF: 4.927, Citation: 1)**

VI. Katona, G., Sabir, F., **Sipos, B.**, Naveed, M., Schelz, Z., Zupkó, I., Csóka, I. (2022). Development of lomustine and n-propyl gallate co-encapsulated liposomes for targeting glioblastoma multiforme via intranasal administration. *Pharmaceutics*, 14(3), 631. **(Q1, IF: 6.525, Citation: 3)**

VII. **Sipos, B**., Csóka, I., Ambrus, R., Schelz, Z., Zupkó, I., Balogh, G.T., Katona, G. (2022). Spray-dried indomethacin – loaded polymeric micelles for the improvement of intestinal drug release and permeability. *European Journal of Pharmaceutical Sciences*, 174, 106200. **(Q1, IF: 5.112, Citation: 3)**

VIII. **Sipos, B.**, Csóka, I., Szivacski, N., Budai – Szűcs, M., Schelz, Z., Zupkó, I., Szabó – Révész, P., Volk, B., Katona, G. (2022). Mucoadhesive meloxicam – loaded nanoemulsions: development, characterization and nasal applicability studies. *European Journal of Pharmaceutical Sciences*, 106229. **(Q1, IF: 5.112, Citation: 4)**

IX. Németh, Z., Csóka, I., Semnani Jazani, R., **Sipos, B.**, Haspel, H., Kozma, G., Kónya, Z., Dobó, D.G. (2022). Quality by Design – driven zeta potential optimisation study of liposomes with charge imparting membrane additives. *Pharmaceutics*, 14(9), 1798. **(Q1, IF: 6.525, Citation: 1)**

X. Katona, G., **Sipos, B.**, Csóka, I. (2022). Risk assessment – based optimization favours the development of albumin nanoparticles with proper characteristics prior to drug loading. *Pharmaceutics*, 14(10), 2036. **(Q1, IF: 6.525, Citation: 1)**

XI. **Sipos, B.**, Budai – Szűcs, M., Kókai, D., Orosz, L., Burián, K., Csorba, A., Nagy, Z.Z., Balogh, G.T., Csóka, I., Katona, G. (2022). Erythromycin – loaded polymeric micelles: in situ gel development, in vitro and ex vivo ocular investigations. *European Journal of Pharmaceutics and Biopharmaceutics*, 180, 81 – 90. **(Q1, IF:5.589, Citation: 2)**

XII. Mardikasari, S.A., **Sipos, B.**, Csóka, I., Katona, G. (2022). Nasal route for antibiotics delivery: advances, challenges and future opportunities applying the quality by design concepts. *Journal of Drug Delivery Science and Technology*, 103887. **(Q1, IF: 5.062, Citation: -)**

XIII. **Sipos, B.**, Katona, G. (2022). Lipid- és fehérjealapú nanomedicinális készítmények a daganatterápiában *Gyógyszerészet*, 66, 633-637

PRESENTATIONS RELATED TO THE SUBJECT OF THE THESIS

A) Oral presentations

I. **Sipos, B.**, Katona, G. (2019). NSAID tartalmú polimer micellák formulációja és vizsgálata, *XXII. Tavaszi Szél Konferencia*

II. **Sipos B.**, Ambrus, R., Csóka, I., Szabó-Révész, P., Katona, G. (2019). Meloxikám tartalmú polimer micellák formulációja és vizsgálata, *XLII. Kémiai Előadói Napok*

III. **Sipos, B.**, Csóka, I., Katona, G. (2020). Formulation and investigation of amphiphilic graft co-polymer based polymeric micelles, *Medical Conference for PhD Students and Experts of Clinical Sciences*

IV. **Sipos, B.**, Katona, G., Csóka, I. (2021). Nose-to-brain applicability of Meloxicam-loaded Soluplus polymeric micelles, *III. Symposium of Young Researchers on Pharmaceutical Technology, Biotechnology and Regulatory Science*

V. **Sipos, B.**, Katona, G., Csóka, I. (2022). Reflection on the regulatory status quo of polymeric micelles as innovative nanocarriers, *Figon & EUFEPS European Medicines Day*

B) Poster presentations

I. **Sipos, B.**, Szabó-Révész, P., Katona, G. (2018). Formulation and investigation of amphiphilic graft co-polymer based polymeric micelles, *12th Central European Symposium on Pharmaceutical Technology and Regulatory Affairs*

II. Katona, G., **Sipos, B.**, Ambrus, R., Csóka, I., Szabó-Révész, P. (2019). Formulation and characterization of Soluplus® based polymeric micelles, *3rd European Conference on Pharmaceutics*

III. **Sipos, B.**, Ambrus, R., Pallagi, E., Szabó-Révész, P., Csóka, I., Katona, G. (2020). Quality by Design: a novel regulatory approach used in the development of nasal polymeric micelles, *Medical Conference for PhD Students and Experts of Clinical Sciences 2020*

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