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Faculty of Pharmacy

Department of Pharmaceutical Technology

Summary of the Ph.D. thesis

APPLICATION OF WET MILLING TECHNIQUES TO PRODUCE MICRONIZED AND NANONIZED DRUG PRE-DISPERSIONS FOR THE DEVELOPMENT OF INTRANASAL FORMULATIONS

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1. INTRODUCTION

Particle design techniques are widely used to modify the physico-chemical and biopharmaceutical properties of active pharmaceutical ingredients (**API**s) (Maghsoodi et al., *J. Pharm. Biomed. Anal.,* 2008). The various size reduction techniques include bottom-up approaches, where micro- or nanoparticles are built up from dissolved drug molecules, and top-down methods, where the raw material is subsequently broken down by using milling methods until micro- or nanosized particles are produced (Ambrus et al., *Int. J. Pharm.,* 2009). Milling belongs among the disintegration procedures. Dry and wet milling can be distinguished. In the wet milling procedure, a sufficiently concentrated dispersion of drug particles in an aqueous or non-aqueous liquid medium is treated. For wet milling, additives are essential, independently of the preparation of micro- or nanoparticles. The choice of stabilizer is specific for each drug candidate and each formulation procedure. Stabilizers help to minimize the agglomeration of suspended particles via electrostatic and steric mechanisms. Through wet milling, the preparation of pre-dispersions is possible, while intermediate solid-state products (powders) can be prepared by means of drying, and the development of liquid or semi-solid formulations (sprays and gels) is feasible directly from pre-dispersions.

Intranasal administration is a possible route for the delivery of drugs to reach the systemic circulation (Prommer and Thompson, *Patient. Pref. Adher.,* 2011). Pharmaceutical formulations delivered intranasally may be powder (Kaye et al., *J. Control. Release,* 2009), gel (Osth et al., *J. Control. Release,* 2002) or liquid (drops and sprays) (Baumann et al., *Eur. J. Pharm. Biopharm.*, 2012) forms. In the case of spray formulations, drugs in dissolved form can achieve the fastest therapeutic effect. However, over 40% of new chemical entities exhibit poor solubility (Beig et al., *Eur. J. Pharm. Biopharm.,* 2012). This problem may be solved through the preparation of a pre-dispersion of a poorly-soluble drug with a suitable technique so as to reach the optimum particle size (**PS**) distribution (**PSD**) for high bioavailability and, following this, the development of a liquid formulation. In order to achieve a systemic effect, intranasal compounds can be mixed with different additives so as to ensure a longer residence time, better mucoadhesion (Horvát et al., *Eur. J. Pharm. Biopharm.,* 2009) and increased permeability (Chunga et al., *Carbohyd. Polym.,* 2010).

It is a major challenge in pharmaceutical technology to find organic solvent-free, cost-effective, time-saving PS reduction techniques which are suitable for preparation of the products (pre-dispersions) of the same quality, built into the process of production of pharmaceutical formulations.

2. AIMS

The aim of my research work was to investigate new possibilities in the field of wet milling techniques through study of the PS reduction effect. Via the preparation of pre-dispersions, different organic solvent-free wet milling techniques were compared (sonication and combined wet milling) and the process parameters affecting the PS and their influence on the physico-chemical and biopharmaceutical properties of drugs were determined. Poorly water-soluble, crystalline, non-steroidal anti-inflammatory model drugs (**NSAID**s) (meloxicam-**MEL** and ibuprofen-**IBU)** were chosen for PS reduction investigations. Intranasal formulations as sprays containing suspended drugs were developed from selected drug pre-dispersions and investigated.

The main steps in the experiments were as follows:

i. The use of acoustic cavitation, an organic solvent-free, static wet milling technique, as a new approach for PS reduction (preliminary studies with IBU and MEL).
ii. Comparisons of the PS reduction effects of static and dynamic sonication as process intensification through use of a factorial design (MEL).
iii. Application of a combination of planetary ball and pearl milling for the production of pre-dispersions of micronized and nanonized MEL.
iv. The development and investigation of intranasal formulations directly from the pre-dispersions containing micro- or nanonized MEL.
v. Creation of a proposal for the production of an innovative intranasal dosage form for pain management through controlled drug delivery.

3. MATERIALS AND METHODS

3.1. Materials

MEL was obtained from EGIS Ltd. (Budapest, Hungary), and IBU from Aldrich Chemie (Deisenhofen, Germany). The milling additives: poly(vinylpyrrolidone) K-25 (**PVP**) was purchased from ISP Customer Service GmBH (Köln, Germany), Poloxamer^{R} and Solutol^{R} from BASF (Ludwigshafen, Germany) and Tween 80^{R} (**Tween**) from Hungaropharma (Budapest, Hungary). Poly(vinyl alcohol) 4-98 (**PVA)** (M_w ~ 27,000) and sodium hyaluronate (**HA)** (M_w = 1,400 kDa) were gifts from Gedeon Richter Plc. (Budapest, Hungary). Mucin (porcine gastric mucin type II) was from Sigma Aldrich (Sigma Aldrich Co. LLC, St. Louis MO, US).

3.2. Methods

3.2.1. Preparation of formulations

3.2.1.1. Preliminary experiments of static sonication for PS reduction of IBU and MEL

A high-power ultrasound device (Hielscher UP 200 S Ultrasonic processor, Germany) operating at 200 W was applied as the *E* input in the sample preparation. The samples were sonicated at room temperature without cooling or by using an ice bath with a standardized temperature at around 18 °C. A range of ultrasonic amplitudes were tested in order to determine the optimum amplitude for 10, 20 or 30 min during the procedures. During the content optimization, different additives (PVP, Poloxamer, Tween, Solutol) were applied (Bartos et al., *Farmacia,* 2014).

3.2.1.2. Preparation of sonicated formulations for the comparison of static and dynamic sonication for reduction of the PS of MEL

MEL was chosen as a NSAID drug for sonication. In each sample, 0.5% of PVP was dissolved in an appropriate volume of water (Table 1). A high-power ultrasound device (200 W) was applied in the sample preparation. The comparison of static and dynamic sonication was carried out using a two-level fractional factorial design of resolution III. Investigated high and low parameter values are presented in Table 1.

Table 1 The applied sonication parameters

	Static sonication	**Dynamic sonication**
Volume (ml)	25; 100	100
Position*	0.25; 0.75	0.25
Pump speed (rpm)	-	50; 100
Concentration of MEL (mg/ml)	2; 18	2; 18
Temperature (ºC)	0; 36	0; 36
Amplitude (%)	30; 70	30; 70
Time (min)	10; 30	10; 30

*Position 0.25: the sonotrode was immersed to 25% of the total depth of the liquid
Position 0.75: the sonotrode was immersed to 75% of the total depth of the liquid

3.2.1.3. Preparation of pre-dispersions with a combination of planetary ball and pearl milling for PS reduction

A wet milling technique (a combination of planetary ball and pearl milling) was employed. Suspensions containing MEL were wet-milled in the planetary ball mill (Retsch PM 100 MA,

Retsch GmbH, Germany). The milling balls were 0.3 mm ZrO_2 beads. 10, 20, 50 and 150 g of beads were applied and milling was carried out without pearls as a benchmark. Suspension sampling was carried out at milling times of 10, 20, 30, 40, 50, 60, 70, 80 and 90 (end of milling) min to perform the PS analysis (**PSA**).

3.2.1.4. Preparation of pre-dispersions for the development of an intranasal formulation

On the basis of preliminary experiments, a modified wet milling technique was employed to prepare the pre-dispersions. 0.5 g of PVA was dissolved in 17.5 ml of phosphate buffer solution (**PBS**) (pH 5.6, the pH of the nasal mucosa) and the resulting solution was used as a dispersant medium in which 2.0 g of MEL was suspended. The suspension was wet-milled in the planetary mill at 400 rpm for 10 or 50 min, using ZrO_2 beads.

3.2.1.5. Preparation of intranasal formulations

The intranasal formulations were prepared directly from the pre-dispersions. 3.0 ml of each pre-dispersion was diluted with PBS (pH 5.6) in order to reach a concentration of 1 mg/ml MEL, and 0.15 g of HA was added; the final formulation therefore contained 5 mg/ml HA. The formulations were stored at 8 ºC in a refrigerator for 24 h. The intranasal viscous liquid formulations containing suspended MEL (referred to below as nasal sprays) were prepared and characterized according to an investigational protocol.

3.2.2. Physical-chemical investigations of solid-state products

Pre-dispersions prepared with different wet milling techniques were dried in order to obtain solid products for physical-chemical investigations.

3.2.2.1. Particle size analysis - PSA

The volume-based PSD of drug in the samples was measured by laser diffraction (Mastersizer 2000) (Malvern Instruments Ltd, Worcestershire, UK). In all cases, the volume-weighted PSD values as D10, D50 and D90 were evaluated (n = 3). The specific surface area (**SSA**) was derived from the PSD data.

*3.2.2.2. Image analysis (scanning electron microscopy - **SEM**)*

After drying of the samples, the shape and surface characteristics of the samples were visualized by using SEM (Hitachi S4700, Hitachi Scientific Ltd., Tokyo, Japan).

*3.2.2.3. X-ray powder diffraction analysis (**XRPD**)*

The physical state of drugs in the samples was evaluated by XRPD. The patterns were produced with an X-ray Diffractometer Miniflex II (Rigaku Co. Tokyo, Japan).

In case of combined milling, the crystallinity of the MEL in dried pre-dispersions was determined semi-kvantitatively via the mean of the decrease of the total area beneath the curve of 2 characteristic peaks.

*3.2.2.4. Differential scanning calorimetry (**DSC**)*

DSC measurements were carried out with a Mettler Toledo DSC 821^e thermal analysis system with the STAR^e thermal analysis program V9.0 (Mettler Inc. Schwerzenbach, Switzerland). Approx. 2-5 mg of pure API or product was examined in the temperature range of 25-300 ºC.

*3.2.2.5. Fourier transform infrared spectroscopy (**FT-IR**)*

FT-IR spectra were recorded with a Bio-Rad Digilab Division FTS-65A / 896 FT-IR spectrometer (Bio-Rad Digilab Division FTS-65A/869, Philadelphia, USA).

3.2.3. Investigation of pre-dispersions for nasal formulations

3.2.3.1. Solubility testing of MEL in the pre-dispersions

The pre-dispersions were stirred with a magnetic stirrer at 25 °C for 24 h and then filtered (0.1 μm, FilterBio PES Syringe Filter) (Labex Ltd., Budapest, Hungary), and the dissolved drug content was analysed spectrophotometrically (Unicam UV/VIS) (Thermo Fisher Scientific Inc., Waltham, MA, USA) (n = 3).

3.2.3.2. Holding time determination of MEL in the pre-dispersions

Pre-dispersions were stored in sealed glass bottles at room temperature (25 ± 1 ºC) for 3 days. The PSD of the MEL in the prepared samples were analysed on the production day (day 0) and after 1, 2 or 3 days storage.

3.2.4. Investigations of nasal formulations

The pH of each nasal spray was determined (Orion 3 star pH-meter), (Thermo Fisher Scientific Inc., Waltham, MA, USA).

3.2.4.1. Rheology and mucoadhesion

Rheological measurements were carried out with a Physica MCR101 Rheometer (Anton Paar GmbH, Graz, Austria). Frequency sweep curves were plotted to determine the viscoelastic character of the samples. The flow curves of the samples were also determined.

The mucoadhesivity of samples was determined on the basis of rheological synergism between the polymer and the mucin. The synergism parameter (bioadhesive viscosity component, η_b) was calculated (Bartos et al., *Int. J. Pharm.*, 2015B).

3.2.4.2. In vitro permeability of MEL

In vitro permeability studies were performed on a vertical Franz diffusion cell system (Hanson Microette Topical and Transdermal Diffusion Cell and Autosampling System) (Hanson Research, Chatsworth CA, USA). The amount of diffused MEL was determined spectrophotometrically (Unicam UV/VIS) (Thermo Fisher Scientific Inc., Waltham, MA, USA). The API flux (**J**) and the permeability coefficient ($\mathbf{K_p}$) were determined. For residual MEL content determination in the donor phase, the membrane was impregnated with simulated nasal fluid (Jug and Bećirević-Laćan, *Comb. Chem. High T. Scr.*, 2007). The remaining MEL amount was determined with an Agilent 1260 RP-HLPC system (QP, DAD, ALS) after 60 min.

3.2.4.3. In vivo study of MEL

A dose of 60 μg MEL *per animal* was administered into the right nostril of 160-180 g male Sprague–Dawley rats (n = 5) via the pipette. Blood samples were withdrawn from the tail vein before and at 5, 15, 30 and 60 min post-dosing. The MEL contents of blood samples were quantitated with an Agilent 1260 HLPC (high-performance liquid chromatography) system. Pharmacokinetic parameters were analysed by means of PK Solver 2.0 software (Zhang et al., *Comput. Meth. Prog. Bio.*, 2010).

4. RESULTS

4.1. Results of preliminary static sonication experiments

During the preliminary study, the effect of static acoustic cavitation in reducing the PS, based on the collapse of the bubbles created by ultrasound waves, was investigated in the cases of IBU and MEL. The smallest particles were produced at an amplitude of 70% (the highest *E* input) with ice cooling in case of both APIs. Although the sonication amplitude was increased (30→70%), the ice cooling prevented the temperature of the suspensions from changing significantly. Increase of the sonication time (10→20 min) had a stronger effect (a small IBU PS reduction and a considerable MEL PS reduction) than the combination of an increased amplitude and ice cooling. Further elevation of the sonication time (20→30 min) did not result in changes in the PSs of IBU and MEL. A sonication time of 20 min was therefore considered to be optimum.

When the effects of different stabilizers were investigated, the suspensions were prepared with a fixed API concentration (300 mg/ml) and fixed parameters (70%, 18 ºC and 20 min). Four different stabilizers were tested in order to check the effect of the nature of the surfactant on the PSD of the drug. In the case of IBU, the smallest PS was achieved with Poloxamer: *D50* ~ 11 μm. In the case of MEL, the most effective PS reduction was achieved with PVP (*D50* = 4 μm). The low stabilizer concentration did not cause any significant variation in the PS.

4.2. Comparison of static and dynamic sonication methods

The effects of static and dynamic sonication on PS reduction were compared. In dynamic sonication, the samples were circulated continuously during the sonication. The most effective process parameters were determined by a factorial design plan for the PSD of MEL. A long sonication (30 min), high amplitude (70%), a high temperature (36 ºC) and a low concentration of MEL (2 mg/ml) proved to play important roles in the sonication procedures and resulted in the most effective particle size reduction (Table 2 and 3).

Table 2 Results of static sonication (pre-dispersion) of MEL

Volume (ml)	Sonotrode position	Concentration (mg/ml)	Temperature (°C)	Amplitude (%)	time (min)	*D10* (µm)	*D50* (µm)	*D90* (µm)
-	-	-	-	-	-	10.82	34.03	75.81
25	0.75	2	36	70	30	1.51	10.16	19.53
100	0.75	2	0	30	30	4.81	23.07	46.88
25	0.25	2	0	70	10	2.75	18.45	42.87
100	0.25	2	36	30	10	5.92	26.52	53.39
25	0.75	18	36	30	10	3.95	19.62	41.51
100	0.75	18	0	70	10	5.19	24.16	46.98
25	0.25	18	0	30	30	3.53	17.12	29.22
100	0.25	18	36	70	30	7.19	20.83	36.62

Table 3 Results of dynamic sonication (pre-dispersion) of MEL

Pump speed (rpm)	Concentration (mg/ml)	Temperature (°C)	Amplitude (%)	Time (min)	*D10*(µm)	*D50*(µm)	*D90*(µm)
-	-	-	-	-	10.82	34.03	75.81
50	2	36	70	30	2.20	14.60	35.02
50	2	0	30	30	4.56	24.22	47.05
100	2	0	70	10	5.70	26.90	51.92
100	2	36	30	10	5.90	26.15	52.20
50	18	36	30	10	4.40	22.69	53.54
50	18	0	70	10	6.27	23.54	46.77
100	18	0	30	30	9.06	29.31	45.58
100	18	36	70	30	2.87	16.73	38.03

4.2.1. PSD and SSA

The PSD of MEL (Figure 1) was determined in the suspensions after sonication. The SSA of the MEL increased as a consequence of acoustic cavitation in both sonication methods and for both suspensions relative to the raw MEL. Sonication of MEL by static process (**MEL Stat.**) led to smaller PSs compared with MEL, sonicated by dynamic process (**MEL Dyn.**)

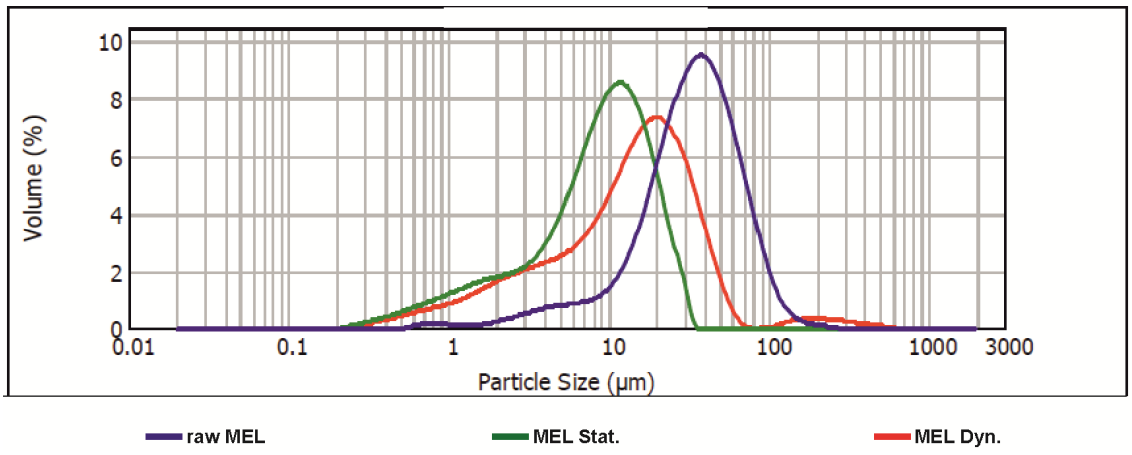

Figure 1 PSDs of raw MEL and sonicated MEL from pre-dispersions

4.2.2. SEM

The crystal habit (particle shape and size) of the pure MEL was changed significantly after the procedure. The raw MEL consisted mainly of angular, prismatic crystals with a broad PSD. The sonication resulted in rounded, micro-sized particles in both cases (Figure 2).

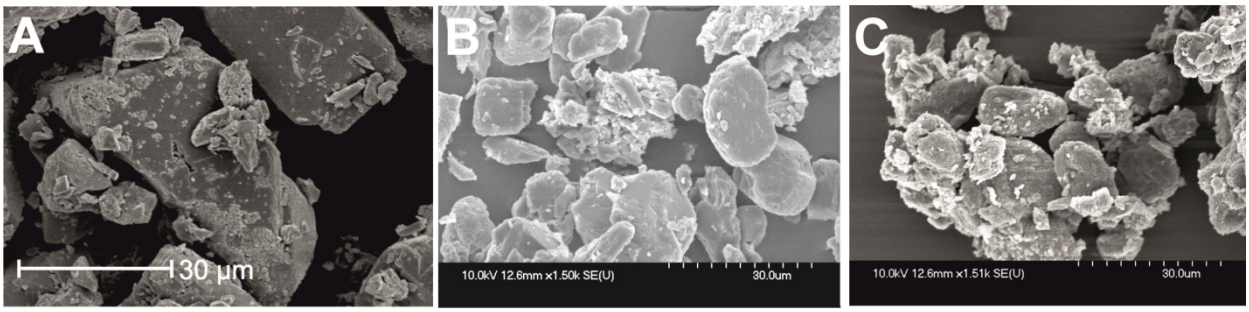

Figure 2 SEM pictures of raw MEL (**A**) and the dried products after static (**B**) and dynamic (**C**) sonication

4.2.3. XRPD

The XRPD pattern of pure MEL demonstrated the crystalline structure, as expected. The characteristic 2θ data were as follows: 13.22, 15.06, 26.46 and 26.67. The raw MEL and the sonicated dried MEL composite in both cases displayed the same XRPD patterns (Figure 3). This means that the crystalline form of the micronized MEL was not changed by the sonication and drying procedures. The intensities of the characteristic peaks were decreased in the case of the sonicated products, due to the reduced PS.

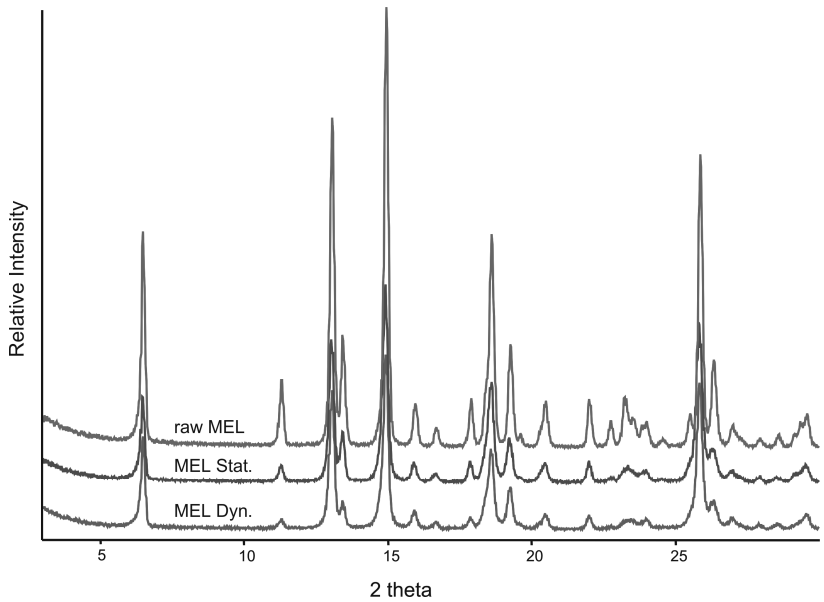

Figure 3 XRPD examination of raw MEL and dried sonicated products

4.2.4. DSC

The DSC curve of the raw MEL revealed a sharp endothermic peak at 259.11 ºC, reflecting its melting point and confirming its crystalline structure. After drying, the DSC curves exhibited the sharp endothermic peak of the MEL at 258.62 ºC in the static case, and at 259.81 ºC in the dynamic case, indicating that the crystallinity of the drug was retained.

4.2.5. Chemical stability (FT-IR)

FT-IR demonstrated that no chemical degradation occurred. The characteristic bands of MEL were seen in all of the curves of the raw MEL and sonicated products, at 3289.76, 1550.04, 1530.36, 1346.73, 1265.88 and 1184.90 1/cm.

4.3. Results of a preliminary study of combined wet milling technique

4.3.1. Effects of milling parameters on PSD

A combination of planetary ball and pearl milling was investigated in the case of MEL (Table 4). In the presence of a stabilizer (PVA), at constant rotation rate (400 rpm), the effects of the milling time, the applied pearl weight and the additive concentration on the reduction of the PS were determined. Depending on the milling time, the PS of the drug could be reduced to the micro-(10 min, *D50* = 1.625 µm) or nanometre (50 min, *D50* = 0.126 µm) range. Increase of the pearl weight above 20 g did not result in the higher effectiveness of milling. The use of a higher concentration (2.5%) of PVA was required to prevent the aggregation of the MEL particles.

Table 4 MEL PSD in pre-dispersions milled with different weights of pearls (0, 10 or 20 g) containing 2.5% PVA solution as dispersant

Milling time (min)	**Without pearls**			**10 g of pearls**			**20 g of pearls**		
	D10 (µm)	**D50 (µm)**	**D90 (µm)**	**D10 (µm)**	**D50 (µm)**	**D90 (µm)**	**D10 (µm)**	**D50 (µm)**	**D90 (µm)**
0	11.40	34.26	73.59	11.40	34.26	73.59	11.40	34.26	73.59
10	10.199	26.616	52.668	0.255	2.934	10.940	0.115	1.625	5.669
20	9.239	25.285	55.202	0.108	1.254	4.775	0.070	0.151	1.951
30	11.207	28.768	54.147	0.080	0.151	2.156	0.068	0.140	1.223
40	8.585	23.848	45.489	0.069	0.146	1.667	0.070	0.135	0.729
50	7.871	24.025	50.346	0.068	0.143	1.280	0.072	0.126	0.271
60	5.203	14.269	27.548	0.068	0.141	1.082	0.069	0.129	0.295
70	5.161	15.047	29.542	0.067	0.135	0.618	0.070	0.131	0.292
80	8.966	25.478	47.930	0.067	0.135	0.538	0.068	0.127	0.288
90	5.805	17.627	34.196	0.069	0.132	0.317	0.068	0.126	0.277

4.3.2. SEM

The SEM images (Figure 4) provided an indication of the morphology of the modified particles. The micronized MEL particles (D50 = 1.625 µm) consisted of aggregations of nanosized particles. The nanonized MEL crystals (D50 = 126 nm) exhibited a regular shape and a smooth surface.

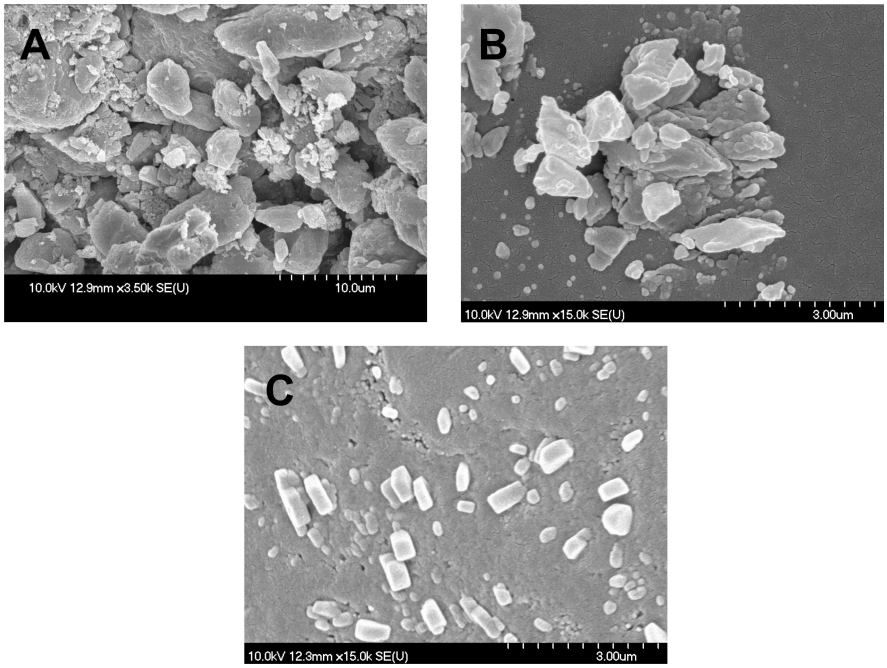

Figure 4 SEM images of raw MEL (**A**), and of MEL from microsized (**B**) and nanosized (**C**) particles containing dried pre-dispersions after milling in PVA-water solution as a dispersant medium

4.3.3. XRPD

The XRPD pattern of the raw MEL demonstrated its crystalline structure, as expected. In the case of MEL-PVA PM, the intensities of the characteristic peaks were decreased due to the PVA. In the course of the milling, a decrease in crystallinity was perceptible, which was determined semi-kvantitatively via the mean of the decrease of the total area beneath the curve of 2 characteristic peaks (at 5.99 and 18.25 2θ). After milling for 10 min, ~ 33% of the drug remained crystalline, and this did not change subsequently (Figure 5).

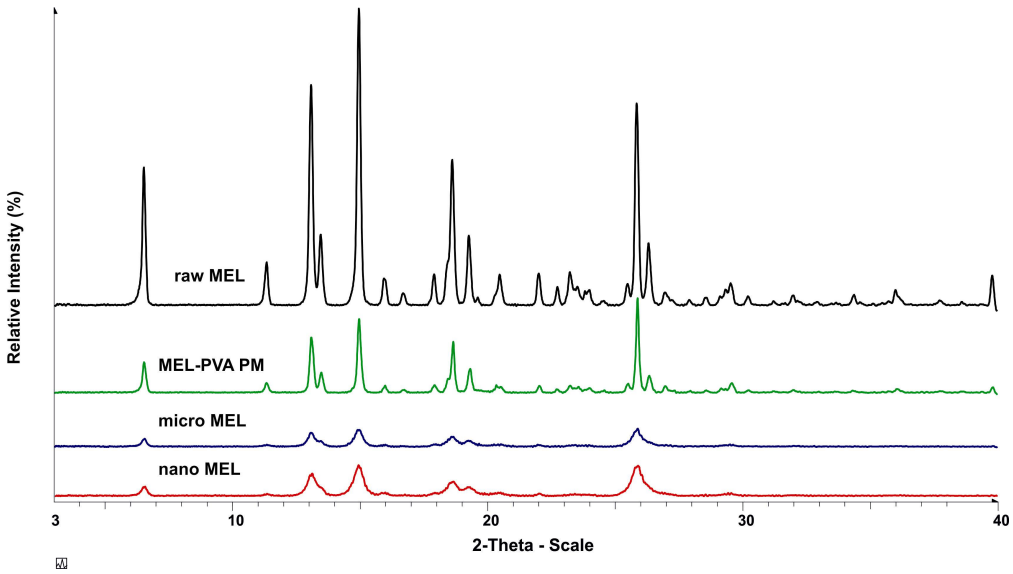

Figure 5 XRPD patterns of raw MEL, MEL-PVA PM, and of MEL from dried, microsized and nanosized particles containing pre-dispersions after milling in PVA-water solution as a dispersant medium

4.3.4. DSC

The DSC curves of the raw MEL and of MEL in the MEL-PVA PM revealed a sharp endothermic peak at 259.11 and 256.57 ºC, reflecting the melting point of MEL and confirming its crystalline structure. After milling and drying, the DSC curves in both cases exhibited the broad endothermic peak of MEL at 239.81 ºC (10 min), and at 240.08 ºC (50 min), indicating that the crystallinity of the drug was decreased.

4.4. Characterization of the intranasal viscous liquid formulations prepared via the combined wet milling technique

4.4.1. Characterization of the pre-dispersions

4.4.1.1. Solubility of MEL in the pre-dispersions

In order to check on the effects of PS reduction on the solubility (S = 6.4 ± 0.2 µg/ml) of MEL in the pre-dispersions, solubility tests were performed at 25 ºC and pH 5.6. Micronization did not result in a change in the solubility of MEL (S = 6.6 ± 0.3 µg/ml). Following nanonization, a slight increase in solubility was observed (S = 9.3 ± 0.5 µg/ml), but the difference did not attain an order of magnitude.

4.4.1.2. Holding time determination

Aggregation did not occur in the pre-dispersions during the first 24 h of storage (micro MEL pre-dispersion: *D90* = 6.462 µm; nano MEL pre-dispersion: *D90* = 0.270 µm). On the second day, however, aggregates were formed in both cases (micro MEL pre-dispersion: *D90* = 1035.340 µm; nano MEL pre-dispersion: *D90* = 695.767 µm), and the number and size of the aggregates increased still further during the third day. To avoid aggregation, the pre-dispersions should be utilized to prepare the formulations within 24 h.

4.4.2. Characterization of the nasal sprays

The pH of the formulations did not change significantly after the addition of HA to the systems (pH 5.5) relative to the pH of the pre-dispersions (pH 5.6).

4.4.2.1. Rheology and mucoadhesion

The viscoelastic characters of the sprays were determined by frequency sweep measurements. The cross-over points of these curves, which are typical for gel-containing hyaluronans, could not be seen (Berkó et al., *Eur. Polym. J.*, 2013). The ratio of *G'* and *G"* indicates the sol state of the samples. The findings can be explained by the pH of the formulations (pH = 5.6) and the low concentration of HA. The different formulations did not indicate changes in the flow characters. The presence of MEL and variation of its PS did not affect the viscosity of the samples.

For the rheological determination of mucoadhesivity, the samples were mixed with mucin (final mucin concentration 5%) and the synergism parameter (η_b) was calculated. The mucoadhesivity values of the HA solution in PBS (pH 5.6) without PVA, of the PVA solution without HA, of the blank and of the three sprays with different MEL PSs were investigated. The highest synergism was observed between the nasal spray containing nanonized MEL and mucin; the mucoadhesivity increased 2-fold as compared with that of the MEL-free blank (Figure 6). The nanosized particles possess an increased adhesiveness to surfaces (Müller et al., *Eur. J. Pharm. Biopharm.*, 2011). On the other hand, nano MEL has a PS similar to those of polymeric molecules such as HA, PVA and mucin chains, which can result in a well-structured complex, and better interactions among the components and it therefore displays more pronounced mucoadhesivity.

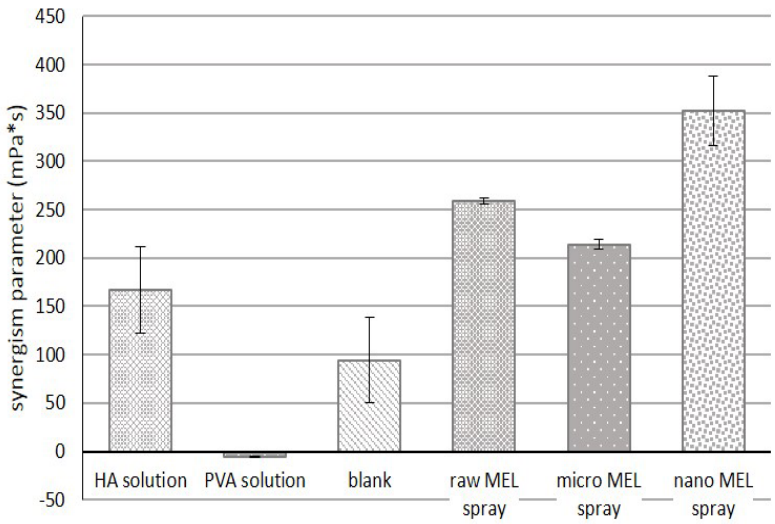

Figure 6 Calculated synergism parameters at a shear rate of 100 1/s of samples

4.4.2.2. In vitro permeability of MEL

The diffusion from the formulation containing MEL nanoparticles was quickest, due to the rapid dissolution of the drug. The diffusion from the nanonized MEL-containing spray started in the first 5 min (Figure 7). The flux and the permeability coefficient were significantly higher in the case of the nasal formulation which contained nanoparticles as compared with the sprays containing micronized or raw MEL (Table 5). The residual MEL content in the donor phase correlated with the decreasing MEL PS of the spray samples.

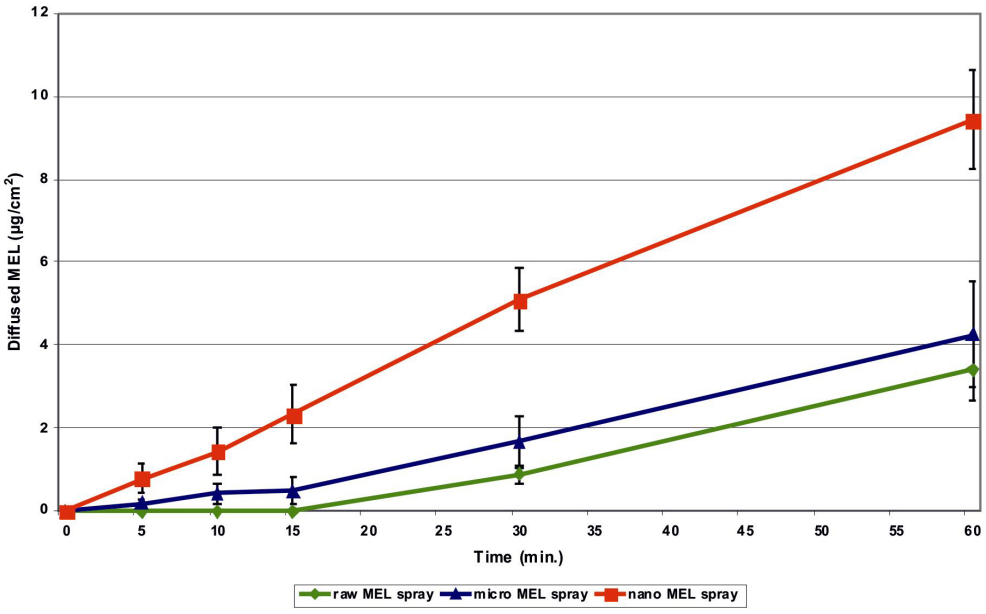

Figure 7 *In vitro* permeability of MEL-containing sprays with different PSs through a synthetic membrane

Table 5 Flux and permeability coefficient values of nasal sprays containing MEL with different PS

	J (µg/cm^2/h)	K_p (cm/h)
raw MEL spray	3.41	0.00341
micro MEL spray	4.25	0.00425
nano MEL spray	9.43	0.00943

4.4.2.3. In vivo study of MEL

The plasma concentrations tended to increase slowly during the initial ~ 30 min, but the 3-fold difference between the sprays containing nanonized or micronized MEL remained during 60 min after treatment (Figure 8). The controlled release of MEL in the case of the nano MEL spray could be explained in terms of the better adhesion and distribution of the nanoparticles and the formation of a well-structured system.

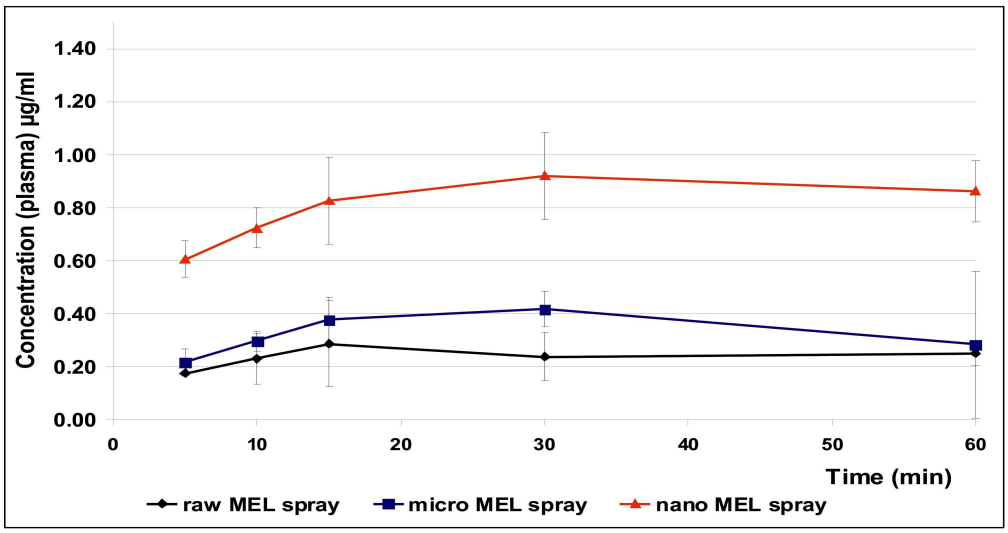

Figure 8 Plasma drug concentration vs. time profiles in rats after intranasal administration of the sprays containing meloxicam with different particle sizes

The AUC is proportional to the amount of drug absorbed during the investigated time interval. The calculated AUC values gradually increased with decreasing PS (the highest AUC was observed for the nano MEL spray).

5. CONCLUSIONS

In this work, effects of different wet milling techniques in reducing the PS were investigated. The results showed that these techniques are suitable for PS reduction and for the preparation of pre-dispersions as intermediates that which are directly applicable for the development of innovative liquid pharmaceutical formulations. The applicability of nanosuspensions in nasal formulations is a new approach in pharmaceutical technology. Drug delivery to the systemic circulation via the nose is considered to be a promising route.

i. The effect of acoustic cavitation in reducing the PS was investigated. During the preliminary study, static sonication was investigated in the cases of IBU and MEL, and the optimum process parameters (temperature, amplitude, sonication period and stabilizers) were determined. It was established that static sonication can be applied to decrease the PS to the micrometre range in the presence of additives.

ii. The comparison of static and dynamic sonication was carried out. The most effective process parameters were determined by a factorial design plan for the PSD of MEL. A long sonication, high amplitude, a high temperature and a low concentration of MEL proved to play important roles in the sonication procedures. Samples sonicated with appropriate parameters were dried and investigated. The SEM images showed that the sonication resulted in rounded, micro-sized particles. XRPD and DSC examinations revealed the crystalline structure of the MEL produced by both sonication methods. FT-IR demonstrated that no chemical degradation occurred. Static sonication is not suitable for scaling-up; this method is recommended primarily for PS reduction in preclinical samples, where the amount of the drug candidate is very small, while dynamic sonication may be suitable for the wet milling of different active substances to prepare pre-dispersions because larger volumes of sample can be used in this method.

iii. A combination of planetary ball and pearl milling was investigated in the case of MEL. Depending on the milling time, the PS of the drug could be reduced to the micro- (10 min) or nanometre (50 min) range. Increase of the pearl weight above 20 g did not result in the higher effectiveness of milling. The use of a higher concentration of PVA was required to prevent the aggregation of the MEL particles. SEM images revealed the aggregation of nano-sized particles, resulting in micronized MEL particles (D50 = 1.625 µm). The nanonized MEL crystals (D50 = 126 nm) exhibited a regular shape and a smooth surface. XRPD and

DSC examinations revealed the change in the crystallinity of MEL. This combined technique is applicable for the production of intermediate (in pre-dispersion form) and (after drying) dried products for additional pharmaceutical formulations.

iv. Of the investigated techniques, the combined milling technique was suitable for the micro- and nanonization of MEL. At pH 5.6, pre-dispersions with different MEL PSs were prepared as intermediates for the design of intranasal liquid formulations with the addition of HA as mucoadhesive agent. Reduction of the MEL PS into the nano range led to increased saturation solubility and dissolution velocities, and increased adhesiveness to surfaces as compared with microsized MEL particles. A linear correlation was demonstrated between the specific surface area of MEL and the AUC. The *in vitro* and *in vivo* studies indicated that a longer residence time and uniform distribution of the nano MEL spray throughout an artificial membrane and the nasal mucosa resulted in better diffusion and a higher AUC. Nanosized MEL may be suggested for the development of an innovative dosage form with a different dose of the drug, as a possible administration route for pain management.

v. It can be concluded that wet milling is applicable for the preparation of pre-dispersions, whereby dosage forms can be prepared in one step. Sonication is suitable for reduction of the PS of drugs to the micro range, but it requires a large amount of dispersion medium, and it is therefore not applicable to obtain intermediate products for the preparation of dosage forms. Metal contamination through degradation of the sonotrode should be borne in mind.

In contrast, because of low need for dispersant medium, the combined method can be used for more efficient milling in comparison with sonication, and it is also suggested for the preparation of pre-dispersions with micro- and nanosized particles, and recommended for the development of PS-controlled intranasal therapeutic systems.

The applicability of a nanosuspension in a nasal formulation is a new approach in pharmaceutical technology, and consequently few data on such systems are available (the intranasal usage of other analgesic NSAID agents (e.g. a ketorolac tromethamine-containing solution) (Li et al., *Int. J. Pharm.*, 2015)). A patent has been granted which describes the nasal application of MEL in solution form (Castile et al., World Intellectual Property Organization patent WO 2005021041, 2005), but there have been no publications on the development of MEL-containing nanosuspensions for nasal application.

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