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Summary of PhD thesis

FORMULATION AND AERODYNAMIC EVALUATION OF CARRIER-FREE DRY POWDER INHALATION SYSTEMS CONTAINING MELOXICAM

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1. INTRODUCTION

The drug delivery by inhalation is a very attractive form of alternative application routs (***Somogyi et al., 2016***) as the lungs offer a lot of advantages both for local and systematic treatment (***Pomázi et al., 2016***). The most of the commercially available inhalation systems are used for local treatment of lung diseases (asthma, COPD, pulmonary fibrosis), but several new approaches reach the clinical trials developed for systematic treatment (e.g. diabetes, cancer) (***Kondo et al., 2018***). Thanks to the anti-inflammatory effect of low water-soluble meloxicam, it could be administered in local pulmonary treatment (***Szabó-Révész, 2018***), but no inhalation systems were prepared yet with higher efficacy than the commercially available inhalation products or using water soluble salt forms of meloxicam for the simplified DPI preparation technology (***Tsubouchi et al., 2000***; ***Pomázi et al.; 2013***). From the inhalation products DPIs have been among the fastest developing inhaler forms in the past decades (***Colombo et al., 2013***). Beside the classical carrier-based formulation (carrier + micronized APIs + additional excipients) (***Zhou et al., 2012***), the carrier-free (APIs + excipients) systems are also gaining the attraction of the new researches (***Pilcer et al., 2010***). These formulations do not need to mix with a bigger separate carrier to deliver the API to the lungs, but the innovative preparation methods and excipients together create the inhalable microparticles (***Healy et al., 2014***). The carrier-free formulations have special morphology/structure and the better aerodynamical properties allow to deposit in the targeted area in the lungs. Many special structured particles can be listed, which can be classified in two main categories: non-porous (spheroids, coated particles, Pulmosol™) (***Yang et al., 2012***; ***White et al., 2005***) and porous formulations (PulmoSphere™, LPP, Nano porous microparticles) (***Cruz et al., 2011***; ***Tsapis et al., 2002***). Both formulation types aimed to reduce the intrinsic cohesion of the particles, increase dispersion and delivery from the inhaler, thus reducing the side ***effects*** of particles deposited in the upper airways (***Hoppentocht et al., 2014***). For the better aerodynamics, formulations involve the use of morphology stabilizers (PVA, sodium hyaluronate and other polymers), aerosolization en-

Abbreviations:

AB – Ammonium bicarbonate; ACI – Andersen cascade impactor; API – Active pharmaceutical ingredient; CFD – Computational fluid dynamics; COPD – Chronic obstructive pulmonary disease; D[0.5] – Geometric diameter; DPI – Dry powder inhaler; DMSO – Dimethyl sulfoxide; EF – Emitted fraction; FDA – Food and Drug Administration; FPF – Fine particle fraction; GSD – Geometric standard deviation; HA – Sodium hyaluronate; IV – Inhaled volume; LEU – L-leucine; LPP – Large porous particle; MMAD – Mass median aerodynamic diameter; MTT – 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; MX – Meloxicam; MXP – Meloxicam potassium; NGI – Next generation impactor; PVA – Poly vinyl alcohol; S – Solubility; SD – Spray dried; $t_{b\text{-}h}$ – Breath-hold time; t_{ex} – Exhalation time; t_{in} – Inhalation time; XRPD – X-ray powder diffraction.

hancers (amino acids, mannitol) (***Moon et al., 2019***), or density modifiers (ammonium bicarbonate, vaporing solvents and other pouring agents) (***Vehring et al, 2008***; ***Martinelli et al, 2017***). The efficacy of the formulations is most commonly tested with Ph. Eur. official *in vitro* assessments (using ACI or NGI) (***Wong et al., 2010***). Beside, novel CFD (***Kerekes et al., 2013***) and *in silico* modelling (***Farkas et al, 2017***), using realistic parameters (anatomical properties, spirometry data of patients, particle properties), is also available for the aerodynamical characterisation of inhalable pharmaceutical and for the composition optimization of novel formulations.

2. AIMS OF THE WORK

- The aim of this PhD work was to develop and investigate innovative **carrier-free DPI** formulations for local pulmonary drug delivery. We aimed to evaluate a **"spray drying from solution" technology**, where MX is dissolved in aqueous solution, without using organic or health harming solvents. This innovative solution formulation was achieved with two feasible strategies:
    - first, with the use of the newly patented meloxicam potassium (**MXP)**, the water-soluble salt form of MX (Egis Pharmaceuticals PLC., Budapest, Hungary,
    - second, with increase of the water solubility of meloxicam (**MX)** with a method that can be incorporated for spray drying.
- We planned to establish the similar effects of MXP and MX with **cell viability assessment** carried out on A459 lung epithelial cancer cell line.
- For the formulations we aimed to use the spray drying method (recommended by the FDA) (***FDA guidance, 2018***), which is easily scalable, controllable and preferred by the industry (Büchi B-191 and Büchi B-290).
- We aimed to evaluate carrier-free DPI formulations with two different spray drying technologies:
    - the planned **non-porous formulations** containing MXP aimed to have a narrow size distribution in the inhalable 1–5 μm range and spherical morphology, while
    - the planned **porous formulations** (LPP) containing MX also aimed to have a narrow size distribution and spherical-like morphology, but with a particle size larger than 5 μm and density lower than 0.20 g/cm^3.
- The aim of the work was also to make a **comparison study** regarding spray drying yield, physicochemical properties (e.g. crystallinity, morphology, density) and *in vitro-in silico* aerodynamic behaviour to establish the advantages and disadvantages of the two formulation methods:

- the *in vitro* aerodynamic properties (EF, FPF, MMAD and GSD) were determined at low inhalation flow rate (30 L/min) with the official **Andersen cascade impactor** (***Eur. Ph. Online 9.6***),
- the *in silico* particle tracking was performed by the validated **Stochastic Lung Model**, where the input data of the simulation were the *in vitro* aerodynamic properties of the particles and the breathing pattern of COPD patients derived from clinical studies.

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Active pharmaceutical ingredients

Both MXP and MX were provided by Egis Pharmaceuticals Plc., Budapest, Hungary (***Mezei et al., 2012***) (***Table I***).

3.1.2. Excipients

In the formulations we used different concentrations of excipients (***Table I***): L-leucine (LEU) (Sigma-Aldrich, USA), polyvinyl alcohol (PVA) (ISP Customer Service GmBH, Germany), sodium hyaluronate (HA) (Acros Organics, Belgium), ammonium bicarbonate (AB) (Sigma-Aldrich, USA).

3.2. Spray drying method for carrier-free DPI production

3.2.1. Sample preparation methods and spray drying

3.2.1.1. Non-porous formulations

We used purified water heated at 70±5 °C as a solvent to increase the solubility of MXP (10 min, 400 rpm, AREC.X heating magnetic stirrer, Velp Scientifica Srl, Italy). The 20 mg/ml MXP solutions were mixed with the appropriate combination of LEU and PVA (***Table I***) (***Chvatal et al., 2017***). Spray drying properties: Büchi B-191 mini spray drier, 140 °C inlet temperature, 75% aspirator rate, 600 L/h gas flow rate, 2.5 mL/min feed pump.

3.2.1.1. Porous formulations

MX at 1.5 mg/mL was dissolved at room temperature in the alkaline aqueous solution (8.0±0.1 pH sodium hydroxide solution, stirring for 2 hours, 600 rpm). LEU and HA solutions were added to obtain final concentrations presented in ***Table I***. AB was dissolved not more than 5 min before the spray drying (***Chvatal et al., 2019***). Spray drying properties: Büchi B-290 mini spray drier, 200 °C inlet temperature, 100% aspirator rate, 414 L/h gas flow rate, 9 mL/min feed pump.

Table I: *The composition of carrier-free DPI formulations in stock aqueous solution prepared for spray drying (mg/mL). *Reference spray dried APIs.*

	Composition of solutions/Used agents			
Non-porous formulations:				
Function	*API*	*Aerodynamic enhancers*	*Structure stabilizer*	–
Agent	**MXP**	**LEU**	**PVA**	–
*MXP-SD**	*20*	–	–	–
MXP/LEU^{20}	20	20	–	–
MXP/LEU^{40}	20	40	–	–
$MXP/LEU^{20}/PVA^{2.5}$	20	20	2.5	–
$MXP/LEU^{40}/PVA^{2.0}$	20	40	2.0	–
Porous formulations:				
Function	*API*	*Aerodynamic enhancers*	*Structure stabilizer*	*Pouring agent*
Agent	**MX**	**LEU**	**HA**	**AB**
*MX-SD**	*1.5*	–	–	–
$MX/LEU/HA^{0.30}/AB^{1.5}$	1.5	0.75	0.30	1.5
$MX/LEU/HA^{0.15}/AB^{1.5}$	1.5	0.75	0.15	1.5
$MX/LEU/HA^{0.30}/AB^{2.0}$	1.5	0.75	0.30	2.0
$MX/LEU/HA^{0.15}/AB^{2.0}$	1.5	0.75	0.15	2.0

3.2.2. Spray drying process efficacy

Spray drying yield was calculated as a percentage by dividing the mass of the powder collected from the container by the initial mass of solids in the solution prepared for drying (n=3). The actual API content (%) after spray drying was quantified by spectrophotometry (UV/VIS, ATI-Unicam, UK) measured at a wavelength of 362 nm (***Chvatal et al., 2019***).

3.3. Cell viability assay

The selected APIs were tested on human epithelial A549 lung carcinoma cells (ATCC®, USA). MTT assay was carried out to examine the possible cytotoxicity of MX and MXP on A549 cells (***Mosmann, 1983***). The cells (10,000 cells/well) were then exposed to different concentrations of MXP and MX for 1 hour. The viable cells were measured *via* Synergy H^1plate spectrophotometer (Biotek®, VT) at 570 nm (***Chvatal et al, 2018***).

3.4. Structural analyses

3.4.1. Identification of active pharmaceutical ingredient

Raman spectroscopy (Thermo Fisher DXR Dispersive Raman with CCD camera, Thermo Fisher Sci. Inc., USA) was applied: laser diode operating at a wavelength of 780 nm; laser power was 6–24 mW at 25 μm slit aperture size on a 2 μm spot size, 6 sec exposure time of 20 scanning in the spectral range of 3300–200 cm^{-1} (***Chvatal et al, 2019***).

3.4.2. Identification of the crystallinity of powders

XRPD spectra were recorded with a BRUKER D8 Advance X-ray diffractometer (Bruker AXS GmbH, Germany) system with Cu Kα1 radiation (λ=1.5406 Å) over 3–40° the interval, Cu target; Ni filter, 40 kV voltage, 40 mA current, 0.1 s time constant, 0.010°angular step.

3.5. Morphology of the particles

Scanning electron microscopy (Hitachi S4700, Hitachi Scientific Ltd., Japan) was used applying 10–15 kV high voltage set and 1.3–13.0 mPa air pressure. Samples were sputter-coated with gold-palladium (Bio-Rad SC 502, VG Microtech, UK) (***Chvatal et al., 2017***).

3.6. Particle size analyses

The volume median diameter of the particles (D[0.5]=the is the diameter where 50% of the distribution is above and 50% is below; referred as geometric diameter) and Span was determined (Malvern Instruments Ltd., UK).

3.7. Tap density measurements

The density of the formulations was measured using a Pharma test PT-TD1 apparatus (Pharma Test Apparatebau AG, Germany) tapped 1000 times (***Eur. Ph. Online 9.6***).

3.8. Aerodynamic characterisation

3.8.1. In vitro aerodynamic assessment

In vitro depositions were established at 30±1 L/min in Andersen cascade impactor (Copley Scientific, UK). After the actuation (t_{in}=4 s, Breezhaler® device) the deposited APIs were quantified by UV/Vis spectrophotometry (ATI-Unicam UV/VIS Spectrophotometer, UK) at λ=362 nm. The aerodynamic properties were calculated based on the absorbance using KaleidaGraph (***Chvatal et al., 2019***): EF (% of the loaded dose reaching the impactor), FPF (% of deposited particles <5 μm), MMAD (diameter of the particle during inhalation) and GSD (distribution of the particles during inhalation) (***Eur. Ph. Online 9.6, Chapter 2.9.18***).

3.8.2. In silico aerodynamic modelling

In order to simulate the realistic breathing of the patients, measured spirometry data of the individuals inhaling through Breezhaler® device were adopted in the Stochastic Lung Model. 7 male and female patients (aged ≥40 years, with a clinical diagnosis of mild to severe COPD) were included in the study ***(Chapman et al., 2011)***. The average values of the measured breathing parameters were the following: *IV*=1.7 L, t_{in}=2.04 s, $t_{b\text{-}h}$=5.0 and 10.0 s (***Farkas et al., 2017***), t_{ex}=3.0 s, mean inhalation flow rate 50.1 L/min (***Chvatal et al., 2017***).

3.9. Stability assay

The most important properties (size, density, aerodynamic properties) determining the aerodynamical effectiveness of the samples were measured in the 1st week after spray drying and after 10 weeks of storage. Samples were stored at room temperature (23±1°C), in a separate desiccator containing cobalt crystals to assess their stability (*Chvatal et al., 2019*). Results will be discussed in each chapter separately.

3.10. Statistical analyses

The statistical analyses were performed with Social Science Statistics Online web page 2019 (*Social Science Statistics Online*). T-test calculation at 0.05 significance level and one-tailed hypothesis (significance difference if $p<0.05$). All reported data are means ± *S.D* of three parallel measurements.

4. RESULTS

4.1. Carrier-free DPI spray drying efficacy

Both non-porous and porous formulations were produced with an acceptable spray drying yield above 60%. Each spray drying was made in triplicate with low variability in the yield (*S.D.*<3). The final API content of the samples was determined too, and it correlated well with the theoretical MXP and MX contents (<10% difference).

4.2. Cell viability assay

In the presence of DMSO, only 10% cell viability was observed. MX and MXP exhibited cytotoxic effect at higher concentrations of 1, 2, 5 and 10 mg/mL compared to the negative control (DMSO) (*Chvatal et al., 2017*). No differences were observed in the effect of raw and spray dried materials at 0.1 and 0.01 mg/mL concentrations (***Fig. 1***). It was clarified that MXP had a similar effect on A549 cells as MX and both can be safely used at 0.1 and 0.01 mg/mL concentrations.

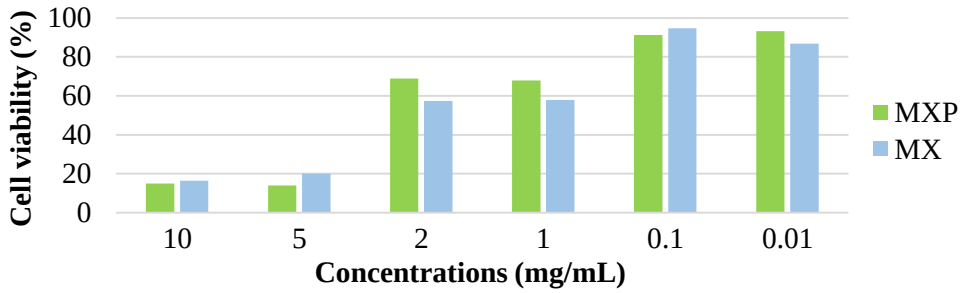

Figure 1: *Cytotoxicity of MXP and MX (Chvatal et al., 2017). Data are presented as mean (n=3), S.D. was less than 0.1% for each concentration.*

4.3. Structural analyses of the carrier-free DPIs

4.3.1. Identification of APIs

There was no difference in the spectra of the spray dried and raw MXP. The Raman spectra of MX-raw show characteristic bands at 1155, 1309, 1540 and 1595 cm^{-1} (**Fig. 2A**). MX-SD shows a difference at 1390 cm^{-1} compared to raw MX, where this band was leaking. These Raman spectra demonstrated that during the dissolution of MX (in pH 8.0±0.1 sodium hydroxide aqueous solution) *in situ* forming of MX sodium salt occurred (***Bio-Rad Laboratories, Inc. SpectraBase; Meloxicam sodium***).

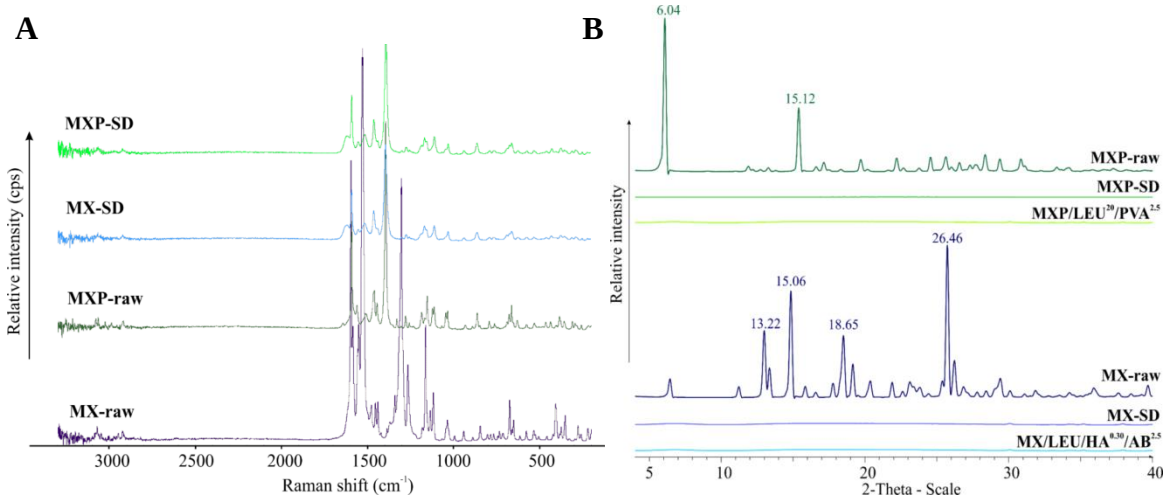

Figure 2*: Raman (A) and XRPD (B) spectra of the raw and spray dried APIs and one of each formulations type.*

4.3.2. Crystal structure

The raw APIs have crystal structure indicated by characteristic peaks of MXP (at 6.04°, 15.35°, 16.51°, 24.52°, 28.33°, 29.40° and 30.94° 2-theta) (***Chvatal et al., 2017***) and MX (at 13.22°, 15.06°, 26.46° 2-theta) (***Chvatal et al., 2019***). The fact that the characteristic peaks of crystalline APIs are missing from the diffractogram of spray dried samples (MXP-SD and MX-SD) indicates that the raw material become amorphous during the spray drying alone (**Fig. 2B**). Semi crystal form was observed in both types of formulations due to the presence of LEU (characteristic peak of LEU at 5.9° 2-theta). The presence of AB, PVA and HA had no effect on the crystalline structure of the samples.

4.4. Morphology

Raw MXP and MX were characterized by large angular crystals unsuitable for pulmonary application. In contrast the excipient-free spray dried samples. In case of non-porous formulations (MX-SD and MXP-SD) had a slightly rough surface and an almost spherical shape for both formulation types. LEU accumulates in the droplet surface during spray drying which shifts the spherical morphology to a shell-formed appearance (***Fig. 3***). The

quick drying core crumples and forms a rough surface which minimized contact area thus can reduce the adhesion between particles. PVA, as well as HA, was used in order to stabilize the structure of the particles: well dispersed particles, with no change in the surface properties were observed. Porous particles exhibit significantly different morphology than non-porous formulations in which AB had the main role. However the surface roughness of porous particles was much lower, slight wrinkles could be detected on the surface of the spherical particles. AB decomposition during the drying "blows" up the strongly wrinkled structure and forms the presented larger sized spheres with internal holes (***Fig. 3***). Between these large sized spheres the cohesion forces could be lowered and high dispersity powders were formed.

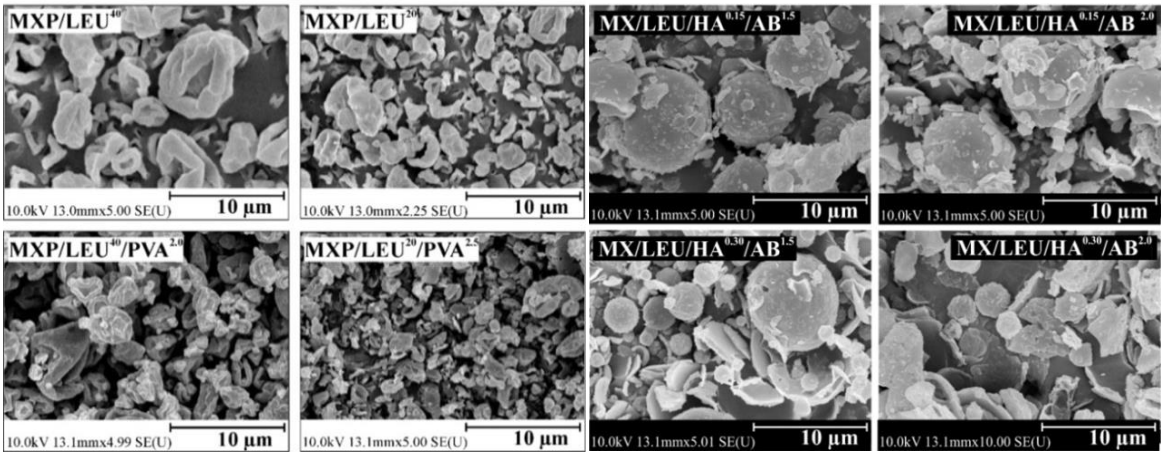

Figure 3*: Electron microscopy pictures of non-porous and porous formulations.*

4.5. Particle size analyses

The medium geometric diameter of the reference spray dried particles (MXP-SD and MX-SD) was around 3 µm (3.0–3.2 µm). (***Table II***). With higher amount of LEU a slight size increasing was detected (with 20 mg/mL LEU 3.1 µm, while 40 mg/mL LEU shows 3.6 µm), but together with PVA this size increase effect was not significant (3.3–3.5 µm). In the case of porous formulation the aimed larger geometric diameter was achieved (larger than 4.9 µm up to 5.7 µm). Geometric diameter was increased significantly up to 5.7 µm with the combination of HA (higher viscosity increased the viscosity of the drying droplets) and of AB (bowling effect caused by the decomposition). Larger amount of HA increased significantly the geometric diameter, while higher AB concentrations had no changing effect on the size. Like non-porous particles, porous particles also had narrow size distribution with Span ≤2.0. No significant differences were detected between the size distribution of the particles measured at 1st week and 10 weeks after of storage.

Table II: *Medium geometric size (D[0.5]) and the size distribution (Span) of the carrier-free DPI formulations. Data are presented as mean ± S.D., n=3. *Reference spray dried APIs.*

	D[0.5] (µm)		Span	
	1st week	***10th week***	***1st week***	***10th week***
Non-porous formulations				
*MXP-SD**	*3.1±0.07*	*3.0±0.03*	*1.3±0.10*	*1.4±0.32*
MXP/LEU^{20}	3.1±0.52	3.2±0.09	1.6±0.73	1.5±0.58
MXP/LEU^{40}	3.6±0.06	3.4±0.15	1.8±0.04	1.8±0.17
$MXP/LEU^{20}/PVA^{2.5}$	3.4±0.24	3.5±0.03	1.8±0.11	1.6±0.15
$MXP/LEU^{40}/PVA^{2.0}$	3.5±0.73	3.3±0.20	1.7±0.10	1.8±0.15
Porous formulations				
*MX-SD**	*3.2±0.08*	*3.0±0.03*	*1.4±0.08*	*1.5±0.10*
$MX/LEU/HA^{0.30}/AB^{1.5}$	5.6±0.73	5.6±0.09	2.0±0.10	1.8±0.11
$MX/LEU/HA^{0.15}/AB^{1.5}$	5.0±0.60	5.0±0.25	1.9±0.22	1.6±0.13
$MX/LEU/HA^{0.30}/AB^{2.0}$	5.6±0.64	5.7±0.35	2.0±0.13	2.0±0.12
$MX/LEU/HA^{0.15}/AB^{2.0}$	4.9±0.60	5.0±0.09	1.9±0.31	1.8±0.25

4.6. Density measurements

The density of the reference spray dried APIs (MXP-SD and MX-SD) was around 0.37 g/cm^3. PVA and HA had no significant effect on the density of the spray dried particles. ***Fig. 4*** clearly shows that in spite of their larger geometric diameter the porous formulations had lower tap density (<0.17 g/cm^3) than the non-porous formulations (≥0.33 g/cm^3). The low density (0.09–0.16 g/cm^3) of porous formulations was in connection with the AB content: due to the bulking properties it forms the porous structure, which may offer better flowability properties. 2.0 and 1.5 mg/mL AB contents had almost the same density decreasing effect (***Fig. 4***).

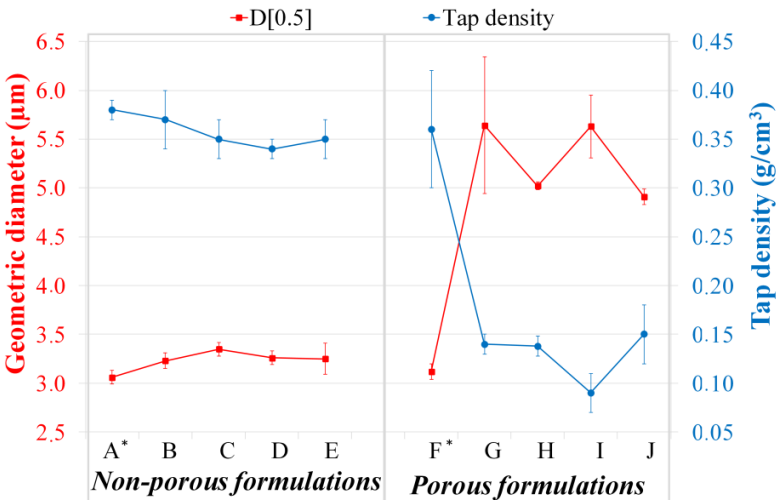

Figure 4: *Comparison of the median geometric diameter (D[0.5]) and tap density of the particles (B: MXP/LEU^{20}, C: MXP/LEU^{40}, D: $MXP/LEU^{20}/PVA^{2.5}$, E: $MXP/LEU^{40}/PVA^{2}$, G: $MX/LEU/HA^{0.3}/AB^{1.5}$, H: $MX/LEU/HA^{0.15}/AB^{1.5}$, I: $MX/LEU/HA^{0.3}/AB^{2}$ and J: $MX/LEU/HA^{0.15}/AB^{2}$). *Reference spray dried particles (A: MXP-SD and B: MX-SD). Data are represented as mean ± S.D., n=3.*

4.7. Aerodynamic assessment

4.7.1. In vitro aerodynamic assessment

The properties of the reference spray dried MXP and MX, with no excipients, had not increased the properties of the commercially available DPIs and may not be efficient for pulmonary treatment (EF ≤59.1% and FPF ≤38.6%) (***Fig. 5***). We detected significant differences between the EF and FPF of the two types of formulations. When non-porous particles were compared, there was no significant difference between LEU and LEU+PVA containing formulations. Although, non-porous particles with higher LEU content (40 mg/mL) increased the EF, different LEU concentrations had no significant effect on the FPF. The lower tap density and porous structure of porous formulations resulted in an improved lung deposition: FPF 54.5–65.8%. The increased, 2.0 mg/mL AB concentration resulted in the highest FPF in case of MX/LEU/$HA^{0.15}$/AB^{2} (measured at the 1st week). Higher AB concentrations had also increased the EF of the porous formulations resulting in > 79.5% drug emission from the inhalation capsules and device. Comparing the porous formulations to each other, it can be concluded that different HA content had no significant effect on the EF or FPF (*Chvatal et al., 2019*).

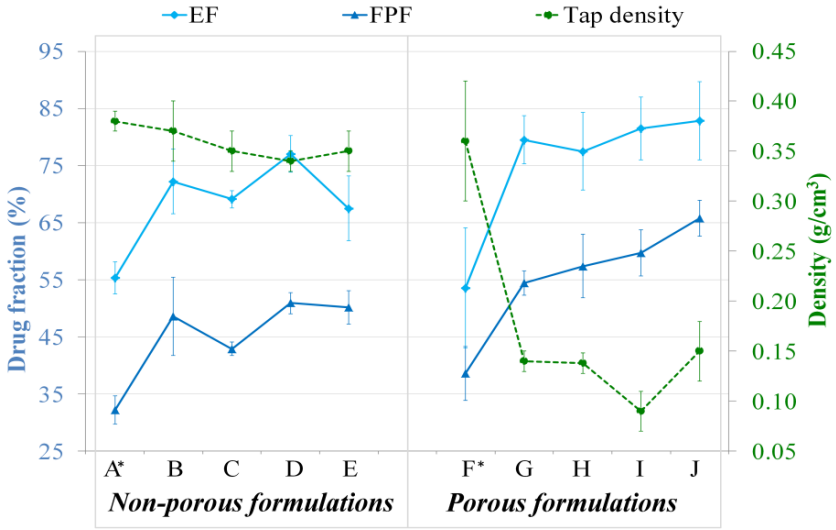

Figure 5: *Comparison of the emitted (EF) and fine particle fractions (FPF) with the tap density of the formulations (B: MXP/LEU^{20}, C: MXP/LEU^{40}, D: MXP/LEU^{20}/$PVA^{2.5}$, E: MXP/LEU^{40}/PVA^{2}, G: MX/LEU/$HA^{0.3}$/$AB^{1.5}$, H: MX/LEU/$HA^{0.15}$/$AB^{1.5}$, I: MX/LEU/$HA^{0.3}$/AB^{2} and J: MX/LEU/$HA^{0.15}$/AB^{2}). *Reference particles (A: MXP-SD and F: MX-SD). Data are represented as mean ± S.D., n=3.*

Figure 6 demonstrates the relevance of the large geometric diameter (>5 µm) and low density (<0.20 g/cm^3) porous particles for lung delivery. Porous and non-porous particles result in the same MMAD values (average 2.6 µm). However, porous particles had larger geometric diameter (D[0.5] ≤4.9 µm) than non-porous formulations The MMAd values drop in the inhalable 1-5 µm size range. In case of non-porous formulations the increasing particle

size resulted in larger MMAD. In case of porous formulations the MMAD was not increasing linearly with the D[0.5]. However, the difference between the D[0.5] and MMAD of non-porous formulations was just 1 µm, porous ones had in average 2.8 µm difference in the same values. Porous formulations with 2.0 mg/mL AB concentration exhibit lower MMAD (2.3–2.4 µm) than those with just 1.5 mg/mL (2.6–2.7 µm). By contrast, there was no significant difference between the MMAD of 0.15 and 0.30 mg/mL HA concentrations.

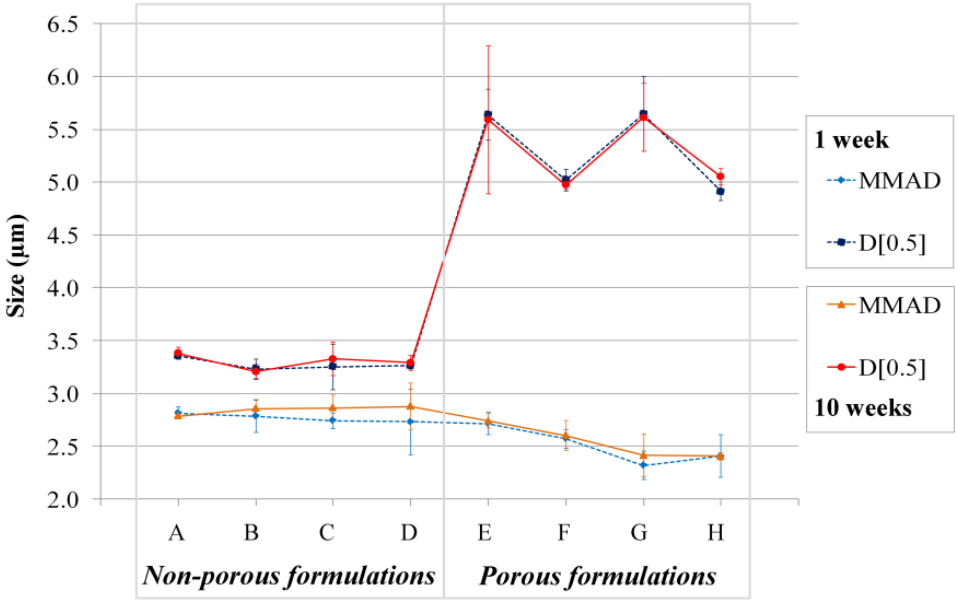

Figure 6: *Comparison of the aerodynamic (MMAD) and geometric diameter (D[0.5]) of the formulations. A: MXP/LEU^{20}, B: MXP/LEU^{40}, C: MXP/LEU^{20}/PVA$^{2.5}$, D: MXP/LEU^{40}/PVA^{2}, E: MX/LEU/HA$^{0.3}$/AB$^{1.5}$, F: MX/LEU/HA$^{0.15}$/AB$^{1.5}$, G: MX/LEU/HA$^{0.3}$/AB^{2}, H: MX/LEU/HA$^{0.15}$/AB^{2}. Data are represented as mean ± S.D., n=3.*

No significant difference was detected in the aerodynamics of the samples when comparing the properties in the 1st week and after 10 weeks of storage. The presented low density porous particles (<0.17 g/cm^3) had better aerosolization properties (EF≥76.1% and FPF≥54.5%) and could reach the lower airways more easily than the smaller but denser non-porous particles (EF≤62.1% and FPF≤41.2%).

4.7.2. In silico aerodynamic modelling

With $t_{b\text{-}h}$=10.0 s higher lung depositions were computed with higher lung (>48.9% for both formulation types) compared to simulations at $t_{b\text{-}h}$=5.0 s. However, $t_{b\text{-}h}$ length had no significant effect on the extrathoracic deposition values (ET) which were in a constant range of 22.2–25.2% ***(Fig. 7***). While, it had no significant effect on the extrathoracic depositions (ET) which were in a constant range of 22.2–25.2%. However, non-porous particles were not reaching 50% deposition after 10 weeks of storage porous particles had constantly above 51% lung depositions with $t_{b\text{-}h}$=10 s. The porous particles had lower exhaled fractions (23.6–27.3%) than the non-porous particles (26.0–27.5%). Several commercially available DPIs

were reported to be tested with the Stochastic Lung Model demonstrating a lower deep-lung deposition compared to the presented DPI formulations (*Jókai et al., 2015*). No significant differences were detected in the *in silico* aerodynamic properties after 1 and 10 weeks of storage.

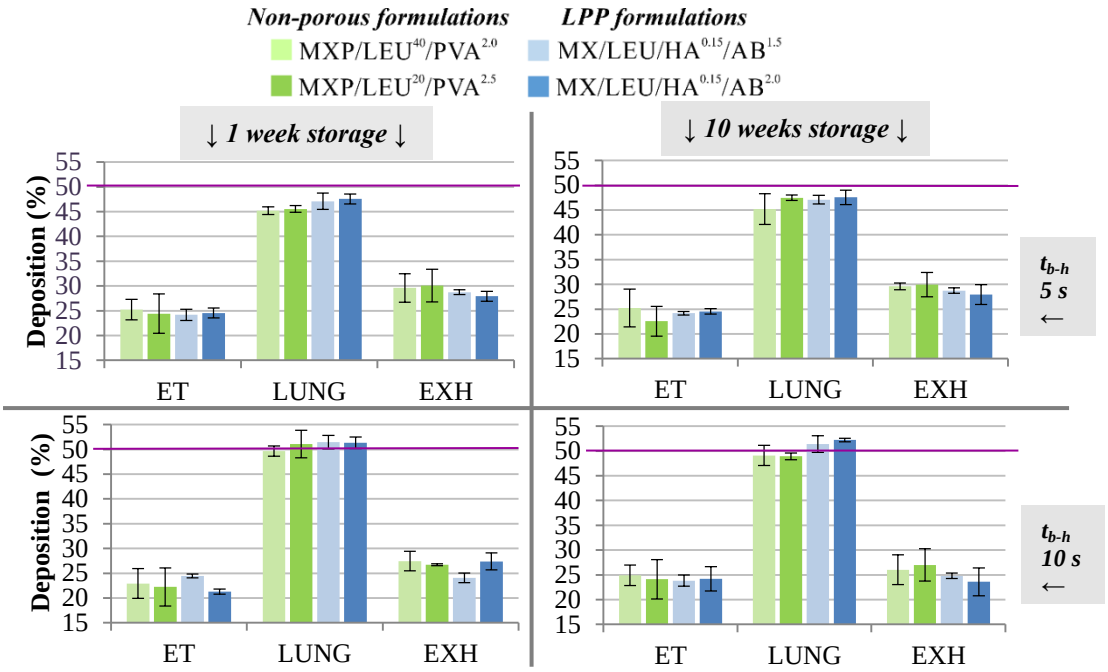

***Figure 7**: In silico modelling results. ET=extrathoracic deposition, LUNG= lung deposition and EXH=exhaled fraction. Data are presented as mean ± S.D., n=3.*

5. CONCLUSION

In accordance with our research goals, carrier-free DPIs were produced with technologies using the novel MXP salt form and applying MX solubility increase (with pH shift). The new "spray drying from solution" technologies eliminate the use of organic solvents for dissolving the active agents. The formulation and analyses protocol was based on a comparison study of the non-porous and porous formulations regarding their spray drying yield (>60% for both technologies), physicochemical properties and aerodynamic behaviour (***Table III***).

It was clarified that MXP and MX had a similar effect on A549 cells and both can be safely used for inhalation up to 0.1 mg/mL concentration for a possible treatment of lung inflammations (e.g. pulmonary fibrosis, COPD).

The combination of *in vitro* and *in silico* assessments presented better aerodynamic behaviour than the present commercially available DPI products. Therefore the prepared formulations could be used for efficient inhalation therapy. The use of *in vitro-in silico*

combination analyses give a precise prediction of *in vivo* behaviour of the formulations thus can be used as a validated tool to characterise the aerodynamics of inhalable pharmaceutics. The most important properties (size, density and aerodynamic properties) determining the aerodynamic behaviour of the presented DPIs were considered stable under the tested conditions (till 10 weeks of storage).

Table III: *Summary of the presented comparison study.*

	Non-porous formulations	**Porous formulations**
Active ingredient	MXP: 20 mg/mL	MX: 1.5 mg/mL
Excipients	LEU: 40–20 mg/mL PVA: 2–2.5 mg/mL	LEU: 0.75 mg/mL HA: 0.15–0.3 mg/mL AB: 1.5–2 mg/mL
Drying yield	60–64%	61–70%
Morphology	shell-like, rough surface	spherical, porous structure
Geometric diameter	3.0–3.8 μm	4.9–5.7 μm
Density	0.30–0.42 g/cm^3	0.09–0.16 g/cm^3
In vitro aerodynamical properties (30 L/min)		
EF	67–78%	77–90%
FPF	42–53%	54–70%
MMAD	2.6–3.0 μm	2.2–2.8 μm
GSD	1.3–1.5	1.7–2.0
In silico aerodynamical properties ($t_{b\text{-}h}$=10 s)		
Extrathoracic	22–24%	21–25%
Lung	48–51%	51–52%
Exhaled	26–27%	23–27%

New approaches and practical relevance of the work:

- A novel "spray drying from aqueous solution" process was developed to design carrier-free MX containing DPIs, which requires no organic solvent and offers the benefits of a green formulation procedure and also a scale up technology with a high spray drying yield (60–70%).
- A formulation and analyses protocol was developed to prepare for the production of new types of DPIs as non-porous particles with MXP, and porous formulation with the pH adjustment of MX.
- Both the non-porous and the porous MX containing formulations had good aerodynamic properties and resulted in better *in vitro-in silico* aerodynamic behaviour than the present commercially available DPI products. Therefore, these novel well controlled MX containing DPI particles could offer new possibilities in the use of non-steroidal anti-inflammatory drugs in inhalation therapy both alone and in combination products for the local treatment of lung inflammation diseases, such as pulmonary fibrosis and COPD.

ORIGINAL PUBLICATIONS RELATED TO THE THESIS

I. **A. Chvatal**, R. Ambrus, P. Party, G. Katona, O. Jójárt-Laczkovich, P. Szabó-Révész, E. Fattal, N. Tsapis. *Formulation and comparison of spray dried non-porous and large porous particles containing meloxicam for pulmonary drug delivery.*
International Journal of Pharmaceutics 559 (2019) 68-75. **IF: 3.862, Q1**

II. **A. Chvatal**, Á. Farkas, I. Balásházy, B. Hopp, P. Szabóné-Révész, R. Ambrus. *Formulaion and in vitro-in silico aerodynamical assesment of carrier-free dry powder inhalation systems*.
Acta Pharmaceutica Hungarica 88 (2018) 3–8. **IF:-**

III. **A. Chvatal**, R. Alzhrani, A. K. Tiwari, R. Ambrus, Piroska Szabó-Révész, S. HS. Boddu. *Cytotoxicity of inhalable dry powders in A549 human lung cancer cell line*.
Farmacia 66 (1) (2018) 172–175. **IF: 1.507, Q2**
Citations: 1

IV. **A. Chvatal**, Á. Farkas, I. Balásházy, P. Szabó-Révész, R. Ambrus. *Structural and aerodynamic evaluation of microcomposites containing meloxicam potassium*.
International Journal of Pharmaceutics 520 (2017) 70–78. **IF: 3.862, Q1**
Citations: 11

V. **A. Chvatal**, E. Benke, P. Szabó-Révész, R. Ambrus. *New strategies of DPI formulations*.
Gyógyszerészet 60 (4) (2016) 197–206. **IF:-**

VI. A. Pomázi, **A. Chvatal**, R. Ambrus, P. Szabó-Révész. *Potential formulation methods and pharmaceutical investigations of Dry Powder Inhalers*.
Gyógyszerészet 58 (3) (2014) 131–139. **IF:-**

PRESENTATIONS RELATED TO THE THESIS

1. 13th Hungarian Aerosol Conference: **A. Chvatal**, Á. Farkas, I. Balásházy, P. Szabó-Révész, R. Ambrus. *Aerodynamical and cytotoxic assessment of spray dried inhalable particles*. Pécs, Hungary 2017 (oral presentation).

2. 12th Ottó Clauder memory competition: **A. Chvatal**. *Formulation and aerodynamical analyses of new generation DPIs.* Budapest, Hungary, 2016 (oral presentation).

3. Richter Gedeon Centenárium Foundation's Session: **A. Chvatal**. *Characterisation of dry powder inhalers containing meloxicam-potassium, using Andersen cascade impactor and Stochastic lung model*. Budapest, Hungary, 2016 (oral presentation).

4. Scientific student conference (TDK): **A. Chvatal**. *Dry powder inhaler formulation of meloxicam-potassium microcomposites*. Szeged, Hungary, 2015 (oral presentation).

5. Scientific student conference (TDK): **A. Chvatal**. *Analyses of co-spray dried microcomposites prepared for inhalable dry powder inhalers*. Szeged, Hungary. 2014 (oral presentation).

6. **A. Chvatal**, P. Party, Á. Farkas, I. Balásházy, R. Ambrus, P. Szabó-Révész, E. Fattal, N. Tsapis. *In vitro and in silico evaluation of carrier-free porous inhalable particles.* P9/4, 12th Central European Symposium on Pharmaceutical Technology and Regulatory Affairs, Szeged, Hungary, 2018 (poster presentation).

7. R. Ambrus, **A. Chvatal**, E. Benke, Á. Zsembery, P. Szabó-Révész. *Development of new generation–formulation containing different water-soluble model drugs for dry powder inhalation.* P-136, 11th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Granada, Spain, 2018 (poster presentation).

8. **A. Chvatal**, R. Alzhrani, R. Ambrus, P. Szabó-Révész, A. K. Tiwari, S. HS. Boddu. *Cytotoxicity and aerodynamical testing of spray dried meloxicam forms for inhalation*. ABS-1557, 6th FIP Pharmaceutical Sciences World Congress (PSWC) Stockholm, Sweden, 2017 (poster presentation).

9. **A. Chvatal**, Á. Farkas, I. Balásházy, R. Ambrus, P. Szabó-Révész. *In vitro and in silico aerodynamical testing of carrier-free DPI formulations*. P118, 2nd European Conference on Pharmaceutics, Novel dosage forms and innovative technologies, Krakow, Poland 2017 (poster presentation).

10. **A. Chvatal**, Á. Farkas, I. Balásházy, R. Ambrus, P. Szabó-Révész. *Characterisation of dry powder inhalers containing meloxicam potassium, using Andersen Cascade Impactor and Stochastic Lung Model assessments*. 22nd Pharmaceutical Technology and Industrial Conference, Siófok, Hungary, 2015 (poster presentation).

11. **A. Chvatal**, A. Pomázi, P. Szabó-Révész, R. Ambrus. *Aerodynamic and structural evaluation of microcomposites containing meloxicam potassium.* P16, 1st European Conference on Pharmaceutics: Drug Delivery, Reims, France, 2015 (poster presentation).

12. **A. Chvatal**, A. Pomázi, P. Szabó-Révész, R. Ambrus. *Aerodynamic and structural evaluation of microcomposites containing meloxicam potassium.* 12th Hungarian Aerosol Conference, Szeged, Hungary, 2015 (poster presentation).

13. **A. Chvatal**, A. Pomázi, R. Ambrus, P. Szabó-Révész. *Stability assessment of dry powder inhalers Containing Meloxicam.* 15th Congressus Pharmaceuticus Hungaricus, Budapest, Hungary, 2014 (poster presentation).

14. A. Pomázi, **A. Chvatal,** R. Ambrus, P. Szabó-Révész. *Analyses Of Co-Spray Dried Microcomposites Prepared For Dry Powder Inhaler Systems.* 15th Congressus Pharmaceuticus Hungaricus, Budapest, Hungary, 2014 (poster presentation).

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