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PhD thesis

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

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PUBLICATIONS RELATED TO THE SUBJECT OF 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.*
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II. **A. Chvatal**, Á. Farkas, I. Balásházy, B. Hopp, P. Szabóné-Révész, R. Ambrus. *Formulaion and in vitro-in silico aerodynamical assessment of carrier-free dry powder inhalation systems*.
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IV. **A. Chvatal**, Á. Farkas, I. Balásházy, P. Szabó-Révész, R. Ambrus. *Structural and aerodynamic evaluation of microcomposites containing meloxicam potassium*.
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VI. A. Pomázi, **A. Chvatal**, R. Ambrus, P. Szabó-Révész. *Potential formulation methods and pharmaceutical investigations of Dry Powder Inhalers*.
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2. 12th Ottó Clauder memory competition: **A. Chvatal**. *Formulation and aerodynamical analyses of new generation DPIs.* Budapest, Hungary, 2016.

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.

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

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

Poster presentations

1. **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.

2. 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.

3. **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.

4. **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.

5. **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.

6. **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.

7. **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.

8. **A. Chvatal**, A. Pomázi, R. Ambrus, P. Szabó-Révész. *Stability assessment of dry powder inhalers containing meloxicam.* 15th Congressus Pharmaceuticus Hungaricus, Budapest, Hungary, 2014.

9. 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.

PRESENTATIONS NOT RELATED TO THE THESIS

1. E. Benke, **A. Chvatal**, R. Ambrus, P. Szabó-Révész. *Formulation of lactose-based dry powder inhalers by surface treatment containing different habit.* 2nd European Conference on Pharmaceutics, Novel dosage forms and innovative technologies, Krakow, Poland, 2017 (poster presentation).

2. **A. Chvatal**, Csa. Bartos, Csi. Bartos, P. Szabóné Révész, R. Ambrus. *Formulation and in vitro characterization of inhalable nano-structured dry powders*. P2B-6, 7th BBBB International Conference, Balatonfüred, Hungary, 2017 (poster presentation).

CONTENT

ABBREVIATIONS

AB – Ammonium bicarbonate
ACI – Andersen cascade impactor
API – Active pharmaceutical ingredient
COX-2 – Cyclooxygenase 2 enzyme
D[0.5] – Geometric diameter
DMEM/F-12 – Dulbecco's Modified Eagle's Medium and Ham's F-12 nutrient mixture
COPD – Chronic obstructive pulmonary disease
DMSO – Dimethyl sulfoxide
DPI – Dry powder inhaler
EF – Emitted fraction
ET – Extrathoracic deposition fraction
EXH –Exhaled fraction
FDA – Food and Drug Administration
FPF – Fine particle fraction
GSD – Geometric standard deviation
HA – Sodium hyaluronate
IV – Inhaled volume
LEU – L-leucine
LPNP – Large porous nanoparticles
LPP – Large porous particle
LUNG – Lung deposition fraction
MMAD – Mass median aerodynamic diameter
MSLI – Multi-stage liquid impinger
MTT – 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide
MX – Meloxicam
MXP – Meloxicam potassium
NGI – Next generation impactor
NIPN – National Institute of Pharmacy and Nutrition
NPMP – Nano porous microparticles
NSAID- Non-steroidal anti-inflammatory drug
NSCLC – Non-small cell lung cancer
Pe – Peclet number
pMDI – Pressurized meter-dose inhalers
PVA – Polyvinyl alcohol
Q – Mean inhalation flow rate
S – Solubility
S.D. – Standard deviation
SD – Spray dried
$t_{b\text{-}h}$ – Breath-hold time
t_{ex} – Exhalation time
t_{in} – Inhalation time
XRPD – X-ray powder diffraction

1. INTRODUCTION

The drug delivery by inhalation is a very attractive form of alternative application routs as the lungs offer a lot of advantages both for local and systematic treatment. In case of local treatment the clinical response is very quick as the drug enters directly at the site of action. However, all the metabolizing enzymes existing in the liver can be found in the lungs, it is considered as low enzymatic environment as it is 5-20 times lower than in the liver. Lower drug concentration is needed for the maximal therapeutic effect which is scaling down form milligrams to micrograms compared to oral doses.

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).

Dry powder inhalers (DPIs) have been among the fastest developing inhaler forms in the past decades. Beside the classical carrier-based formulation (carrier + micronized APIs + additional excipients), the carrier-free (APIs + excipients) systems are also gaining the attraction of the new researches. 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. The carrier-free formulations have special morphology/structure and the better aerodynamic 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™) and porous formulations (PulmoSphere™, Large porous particles, Nano-porous microparticles). 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. For the better aerodynamics, formulations involve the use of morphology stabilizers (PVA, sodium hyaluronate and other polymers), aerosolization enhancers (amino acids, mannitol), or density modifiers (ammonium bicarbonate, vaporing solvents and other pouring agents). The efficacy of the formulations is most commonly tested with Ph. Eur. official *in vitro* assessments (Andersen cascade impactor or Next generation impactor). Beside, novel *in silico* modelling, using realistic parameters (anatomical properties, spirometry data of patients, particle properties), are also available for the aerodynamic characterisation of inhalable pharmaceuticals and composition optimization of novel formulations.

2. LITERATURE BACKGROUND OF THE RESEARCH WORK

2.1. DPI characterisation

For pulmonary drug delivery nebulizers, pMDIs and DPIs are the commonly used oral inhalation products (***Hindle, 2008***; ***Traini et al., 2013***; ***Moon et al., 2019***). In recent decades the use of DPIs can be observed thanks to their advantages:

- stability: the dry powder form is more stable and easier to handle than liquid forms,
- ease of use: overcomes the inhalation coordination issues associated with the other inhalation forms (pMDI),
- environmental friendly: propellant-free, and
- safety: normally DPIs do not require sterilization (***Anderson, 2005***).

For DPI formulation two main routes can be described: carrier-based and carrier-free formulation methods (***Fig. 1***). A carrier-based formulation is made by blending a micronized (1–5 μm) API with 50–200 μm sized inert carrier (e.g. lactose, mannitol). The appropriate API distribution on the surface of the carrier results in a more easily metered (with reduced cohesion) product with sufficient bulk for inhalation (***Colombo et al., 2013***). The product is separated during inhalation: the API reaches the lungs, while the large sized carrier deposits in the upper airways (***Hoppentocht et al., 2014***). When a carrier-free formulation is prepared, the API is formulated with the appropriate excipients together creating an inhalable microcomposite, without any separate carrier (***Chvatal et al., 2016***). The carrier-free formulation is aimed to reduce the intrinsic cohesion of the particles, increase dispersion and delivery from the inhaler.

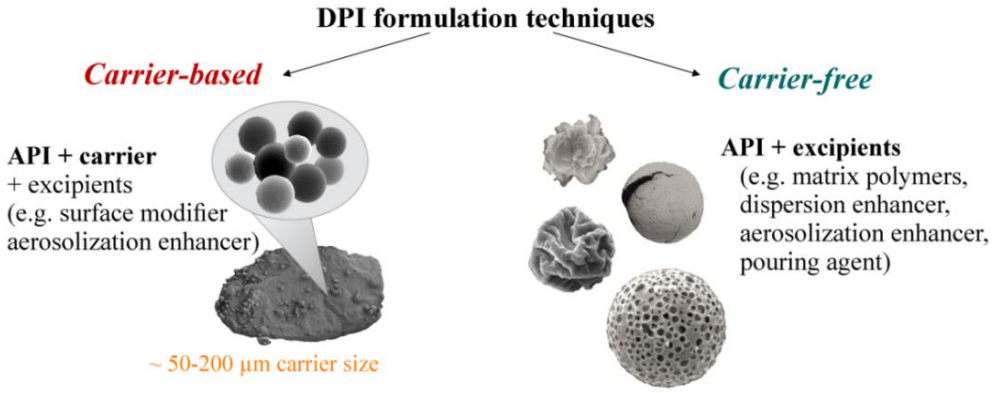


Figure 1*: Classification of DPI products based on the formulation type: carrier-based and carrier-free formulations (**Chvatal et al., 2016**).*

Although the main researches focus on the formulation of high efficacy carrier-free DPIs, just a few products were approved commercially (***Table I***). The task of pharmaceutical technology is the development of innovative formulations for carrier-free products passing clinical trials and reaching the patients. These new carrier-free formulations are mainly used for local treatment (e.g. in cystic fibrosis, COPD, pulmonary inflammation caused by bacterial infections, lung cancers) and might as well be used for systemic therapy provided that the active agent incorporated is well-absorbed from the mucous membrane of the airways (***Weers et al., 2015***; ***Kondo et al., 2018***). The new formulations under development involve different types of active drugs, such as antibiotics, proteins, peptides or other large molecules, either soluble or insoluble in water (***Belotti et al., 2015***; ***Healy et al., 2014***; ***Lam et al., 2013***).

Table I: *Examples for commercially available DPI* products (***NIPN online page***).

Product/Inhaler/Company	**API**	**Main excipients**	**Indication**
Carrier-based formulations			
Bufomix Easyhaler *(Orion Corporation)*	budesonide + formoterol	lactose carrier	asthma
Spiriva Handihaler *(Boehringer Ingelheim International)*	tiotropium-bromide	lactose carrier	COPD
Onbrez Breezhaler *(Novartis Europharm)*	indakaterol	lactose carrier	COPD
Foster NEXThaler *(Chiesi)*	beclomethasone dipropionate + formoterol hemifumarate	lactose carrier + magnesium stearate	COPD, asthma
Seretide Diskus *(GlaxoSmithKline)*	salmeterol xinafolate + fluticasone propionate	lactose carrier	COPD
Carrier-free formulations			
Afrezza Dreamboat *(MannKind Corp)*	insulin	diketopiperazine fumarate	diabetes
TOBI Podhaler *(Novartis)*	tobramycin	DSPC + perflubron	CF with *Pseud. Aerug.* infection
Pulmicort Turbuhaler *(AstraZeneca)*	budesonide	-	COPD, asthma
Brycanil Turbuhaler *(AstraZeneca)*	terbutaline	-	asthma

Abbreviations in the table*: DSPC: 1,2-distearoyl-sn-glycero-3-phosphocholine; perflubron: perfluorooctyl bromide; Pseud. Aerug.: Pseudomonas Aeruginosa*

2.1.1. Advantages of carrier-free DPI

Carrier-free DPIs have the benefit to overcome carrier-associated formulation and technological problems (***Zhou et al., 2012***):

- *Strong or weak binding*: The aerosolization of the carrier-based formulations depends on the characteristics of the micronized API + larger carrier (morphology of the particles, moisture uptake and aggregation) and the link between the two. If the link is weak, the API detaches before inhalation (during storage), while with strong binding the API remains on the surface of the carrier and together deposit in the upper airways. Both processes reduce the effective deposition as the API will not reach the site of action (***Pilcer et al., 2010***). The aerosolization properties of carrier-free microcomposites will depend just on the characteristics of the microcomposites (containing API and the needed excipients), as these particles form a complex structure together (microcomposite).
- *Low inhalation flow rate*: Without the appropriate inhalation manoeuvre (inhalation time and flow rate, breath-hold time) the turbulent air flow may not be enough to detach the drug particles from the surface of the carrier and together they deposit in the upper airways, thus causing side effects. Thanks to the better aerosolization properties of carrier-free formulations, particles follow the inhalation flow till the lower airways even at lower inhalation or insufficient breath-hold time (***Pilcer et al., 2010***; ***Farkas et al., 2017***).
- *Sugar as carrier*: The carriers used for DPI preparations larger than the respirable range (50–200 μm) deposited in larger amount in the mouth and upper throat can cause candida infections (***Pilcer et al., 2010***). However, carrier-free DPIs have no separate detachable fraction the API + excipients reach the site of action together.

2.1.2. Carrier-free DPI formulation techniques

In all carrier-free formulations, special additives are applied in the particle designing phase to make the microcomposites easy to handle during manufacturing, to implement an enhanced aerosolization property for the inhalation and to reduce the cohesion forces between particles without using large carriers (***Healy et al., 2014***; ***Weers et al., 2015***). The most important advantages of these carrier-free DPI formulations are the low adhesion, special morphology and low density of the particles, which improve the aerosolization properties, thus the lung deposition and the efficacy of the powders.

Although several carrier-free formulation techniques can be listed, the basic classification is non-porous or porous formulations (***Fig. 2***).

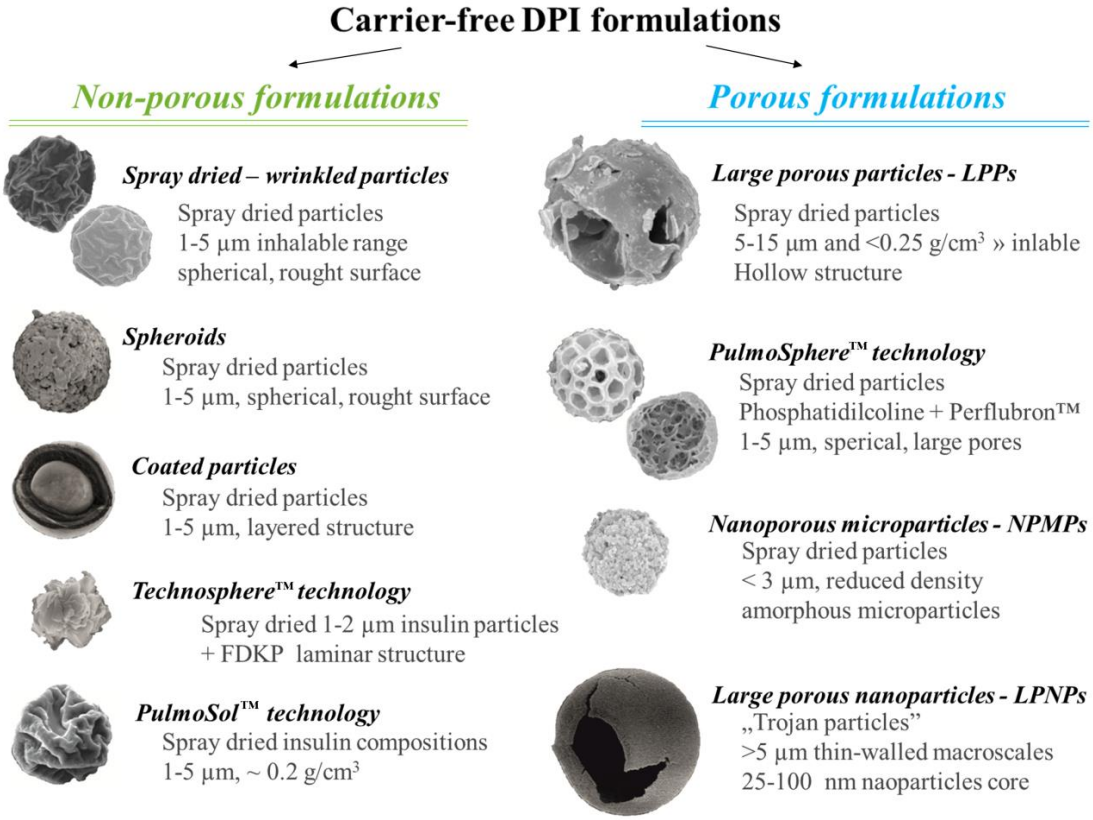


Figure 2: *Classification of the most commonly used carrier-free formulations (**Healy et al., 2014**; **Chvatal et al., 2015**).*

2.1.2.1. Classification of carrier-free DPI formulations

As mentioned above, the carrier-free formulations can be divided into two main parts: non-porous (spray dried particles (***Lechuga-Ballesteros et al., 2008***; ***Islam et al., 2008***), spheroids (***Yang et al., 2012***), coated particles (***Hoe et al., 2014***), Technosphere™ (***Pfützner et al., 2005***), PulmoSol™ technologies (***White et al., 2005***)) and porous formulation methods (LPPs (***Patel et al., 2012***; ***Meenach et al., 2012***), PulmoSpheres™ (***Weers et al., 2014***, ***Duddu et al., 2002***), NPMPs (***Lorraine et al., 2009***; ***Amaro et al., 2011***), LPNPs (***Pison et al., 2006***; ***Tsapis et al., 2002***) (***Fig. 2***).

Non-porous formulations are generally dense structured particles with geometric size in the inhalable 1-5 µm range. Innovative formulations by spray drying can be produced to create the special structure and the appropriate morphology of the API both for carrier-based and for carrier-free formulations (***Tsapis, 2014***). The formulation of stabile

structured porous formulations like LPPs may be a challenge for pharmaceutical technology, to formulate larger than 5 µm geometric sized particles (5-15 µm) with lower than 0.25 g/cm^3. Besides better aerodynamics, porous particles are less phagocyted and cleared from the lungs, which makes them advantageous for local treatment *(**Gervelas et al., 2007**)*. Pouring agents will increase flowability with a hollow/porous structure forming effect, by evaporating from the wet droplet and creating holes in the dry particles. Many formulation strategies have been developed for both non-porous and porous formulations. Most of them utilize bottom-up techniques, such as supercritical solvent technology, spray freeze drying or spray drying (***Gradon et al., 2014***). The newest DPI researches report about several high potency carrier-free formulations prepared with spray drying. It has been widely used in DPI production because it is scalable and offers an easily controlled particle formulation (***Sarrate et al., 2015***). It is also mentioned by FDA as process for achieving a uniform distribution of the drug substance in the formulation (***FDA guidance, 2018***). Spray dried particles become micronized and obtain appropriate morphology for inhalation. During spray drying, the density and the porosity of the particles can be modified, too. Using agents with high diffusion coefficient (when *Pe* <1) or pouring agents (ethanol and other volatile liquids or ammonium bicarbonate) reduces the density of dried particles (***Feng et al., 2011***) (***Fig. 3***). *Pe* number is dependent on the evaporation rate and the diffusion coefficient of the primal solution as described in the paper of Vehring et al: during drying, the API + excipients distribution depends on the diffusion rate in the droplet (***Vehring at al., 2008***; ***Weers et al, 2014***).

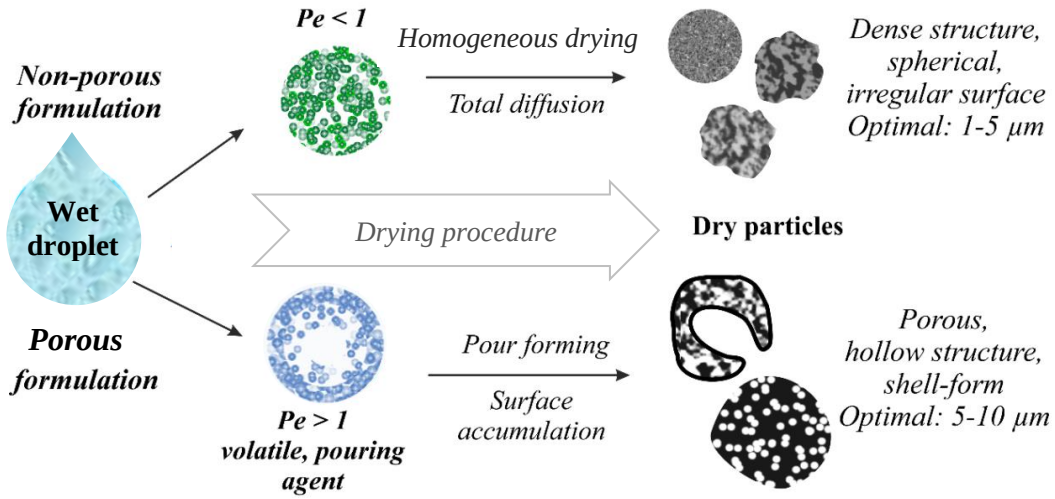


Figure 3: *Droplet drying mechanisms affecting the porosity, density and shape of the carrier-free DPI particles.*

2.1.2.2. Excipients and technologies used for carrier-free DPI production

The physicochemical, and microbiological properties of the excipients should be within an appropriate limit, range, or distribution to ensure the desired product quality (***Buttini et al., 2018***); for example, the physicochemical properties of the APIs and excipients, and their interactions (e.g., densities, amorphous or crystalline forms, flow properties, adhesive and cohesive properties) (***FDA guidance, 2018***). To reach the required aerosolization performance, many types of excipients are used for the formulations (***Moon et al., 2019***) (***Table II***).

Table II: *Examples for the most widely used excipients for carrier-free DPI formulations.*

Type	Examples	Function	Reference
Amino acids	D or L-leucine Trileucine Isoleucine Glycine Alanine	Dispersity increase Aerosolization enhancer	***Minne et al., 2008*** ***Prota et al., 2011*** ***Simon et al., 2016***
Polymers	PLGA Sodium hyaluronate Chitosan PVA PVP PEG	Matrix Coating Stabilizing agent For sustained release	***Tewes et al., 2010*** ***Yang et al., 2012*** ***Iskandar et al., 2009*** ***Martinelli et al., 2017***
Lipids	DPPC PC DMPC DSPC	Matrix Coating Absorption enhancer Encapsulation	***Scalia et al., 2013*** ***Pomázi et al., 2013*** ***Mehta, 2016*** ***Lam et al., 2013***
Poring agents	Ethanol Ammonium carbonate or bicarbonate Perfluorooctyl bromide	Density decrease Aerosolization enhancer For porous particles	***Dellamary et al., 2000*** ***Ogienko et al., 2017*** ***Weers et al., 2014***
Other excipients	Mg stearate	Moisture protection	***Parlati et al., 2009*** ***Yu et al., 2018***
	Chitosan	Fine carrier	***Makhlof et al., 2010***
	DKPF	Fine carrier	***Angelo et al., 2009*** ***Kaur et al., 2008***

Abbreviations in the table: *Poly(lactic-co-glycolic acid) (PLGA), Polyvinyl alcohol (PVA), Poly-vinyl-pyrrolidone (PVP), Polyethylene glycol (PEG), Dipalmitoyl phosphatidylcholine (DPPC), Phosphatidylcholine (PC), Dipalmitoyl phosphatidylcholine (DMPC), Diketopiperazine fumarate (DKPF), perfluorooctyl bromide (perflubron), Distearoylphosphatidylcholine (DSPC)*

Leucine analogues are among the wieldiest used additives to increase the aerosolization behaviour of the particles (***Vanbever et al., 1999***; ***Prota et al., 2011***). Leucine increases the dispersity of the particles and can also form thin layers on the surface of an active ingredient to decrease the surface energy (cohesive and adhesive forces) between particles (***Vehring et al., 2008***; ***Raula et al., 2010***). It was also demonstrated that L-leucine (above 20 w/w% content) recrystallizes during spray drying, covering the surface of the particle and protecting it against moisture (***Li et al., 2016***).

Polymers are used both for non-porous and porous particle formulations for many reasons. The suitably chosen polymer can function as a matrix, such as the widely used PLGA (poly-lactic-co-glycolic acid), to carry the active ingredient(s) (***Yang et al., 20012***; ***Liang et al., 2015***). However, PLGA can only be processed in organic solvent, the newest researches presented no or very low cytotoxic effect of these formulations (***Fatal at al., 2009***). PVA and PVP were used to cover the surface of the particles and decrease the cohesion forces between the particles which tend to agglomerate (***Pomázi et al., 2013***). Biodegradable polymers, such as sodium hyaluronate (biodegradable in the alveolar macrophages), are also used in the carrier-free DPI formulation techniques. Hyaluronate can be used in pulmonary drug delivery for drug targeting, due to its mucoadhesive and structure stabilizing effect (***Cantor et al., 2004***; ***Zhou et al., 2003***). It is also can be used as API: innovative formulations by spray drying are used to implement high efficacy for dry powder hyaluronate products (***Martinelli et al., 2017***).

An innovative trend in carrier-free formulation, besides non-porous particles, is porous particle formulation. Particles with low density have better flowability and can be delivered to the lung more easily with higher deposition *(**Bosquillon et al., 2001***; ***Watts et al., 2013**)*. The value is not clearly established, but most of the studies consider the DPI as ”low density” from a tap density around 0.4–0.1 g/cm^3 or lower *(**Ogienko et al., 2017**; **Ógáin et al., 2011**; **Cruz et al., 2011**)*. With low density formulation the geometric size can range from 5-15 μm (above the easily inhalable size range), as low density and special morphology result in an aerodynamic diameter between 1–5 μm, suitable for inhalation and for the appropriate lung deposition (***Healy et al, 2014***). Special structured porous particles can be obtained by using a porogen agent (e.g. volatile liquids, ammonium carbonate or bicarbonate) in primary solutions or emulsions (***Edwards et al., 1998***). The porogen evaporates quicker than the dispersion phase dries, leaving holes in the interior of the larger sized particles (***N’Guessan et al., 2018***; ***Pham et al., 2013***).

2.2. Determinants of effective inhalation and carrier-free DPI deposition

Besides the physiological advantages of the lungs, several other physical properties have a significant effect on the final effectiveness of the local therapy. The caused influence should be taken into consideration during the particle formulation process. Determinants of effective inhalation (*Yang et al., 2014*; *Farkas et al, 2015*; *Demoly et al., 2014*):

- aerodynamics of the powder (flowability, aggregation),
- particle characteristics (size, morphology, density, hygroscopicity),
- breathing pattern (inhalation, exhalation, breath-hold time, inhalation flow rate) and
- inhalation device type (multi or single dose, resistance of the device, structure).

2.2.1. Aerosolization dynamics of the inhaled DPI particles

Particles reaching the airways follow different deposition mechanisms influenced by their size, shape, density etc. These mechanisms are impaction, sedimentation and Brownian diffusion (***Fig. 4***). In the first 10 generations of the airways (upper airways) impaction is the most significant deposition mechanism. It usually influences the early deposition of the particles larger than 10 μm in the extrathoracic areas. Impaction is also influenced by the velocity of the inhalation flow (*Colombo et al., 2013*). Particles between 1–10 μm settle by a gravity-dependent progress, called sedimentation. Sedimentation is the most important mechanism of effective drug inhalation and deposition as it can be influenced (besides the particle characteristics) by the inhalation mechanism The longer the breath-hold time is, the longer time the particle has for deposition controlled by gravity forces. While particles smaller than 1 μm move with an irregular motion (Brownian diffusion) caused by the collision of the small particles and the air molecules (*Chvatal et al., 2016*).

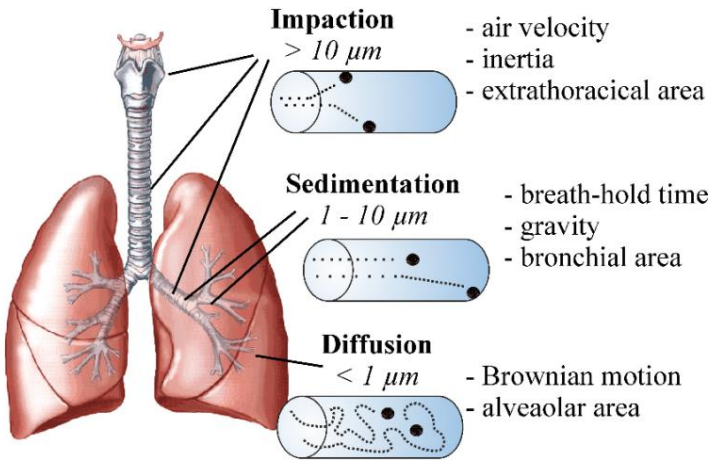


Figure 4*: Deposition mechanisms in the airways (Chvatal et al., 2016).*

2.2.2. Aerodynamic testing of DPIs

2.2.2.1. In vitro aerodynamic measurements

By aerodynamic properties we mean the flowability characteristics of the inhaled particles: FPF and MMAD are the most widely used *in vitro* values. FPF is defined as the mass of the active ingredient consisting of particles having an aerodynamic diameter of less than 5 μm, as under this size range particles reach and deposit in the lower airways well (***Eur. Ph. Online 9.6***). The aerodynamic diameter is influenced by the inhalation flow rate, density, size and shape of the particle. It usually differs from the geometric diameter, thus the real size of the particle during inhalation is expressed with the MMAD. For an inhalable and well deposited powder, the MMAD should be in the 1–5 μm size range (***Colombo et al., 2013***). Besides FPF and MMAD, the fine particle dose (FPD), EF or emitted dose (ED), and GSD can also be calculated which give useful information about the flowability of the powder (***Hamishehkar et al., 2012***). A larger GSD implies a longer large particle size tail in the distribution (***Musante et al., 2002***), while GSD for an ideal distribution would be 1 and indicates a uniform aerosol (***Fig. 5***).

Different statistical calculations and interpolation methods can be used for the determination of these aerodynamic properties. The widely used one is the interpolation from a log-probability-scale plot: cumulative percentage undersize against the log of the cut-off diameter on each stage (***Colombo et al., 2013***) (***Fig. 5***).

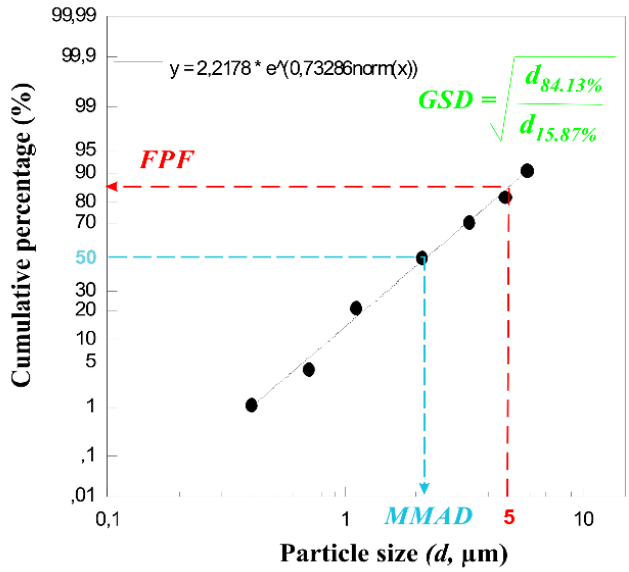


Figure 5*: Collection-efficiency curve used for the aerodynamic property calculation.*

In the European Pharmacopeia four multi-stage impactors are listed as official for aerodynamic property analysis (*Eur. Ph. Online 9.6*), namely the Twin-stage impinger - glass impinger (apparatus A), the MSLI (apparatus C), the ACI (apparatus D), and the NGI (apparatus E) (***Fig. 6***) (*Wong et al., 2010*). The glass impinger is a simple device designed for 60 L/min inhalation flow rate simulation and it is quite inefficient in the determination of aerodynamic size distribution. The most effective are the ACI and NGI which function with a vacuum pump and flow meter to create the optimal flow rate through the stages. The same induction port can be used for all, and they are suitable for a flow rate in the range of 28.3–100 L/min, modelling different inhalation profiles (*Colombo et al., 2013*).

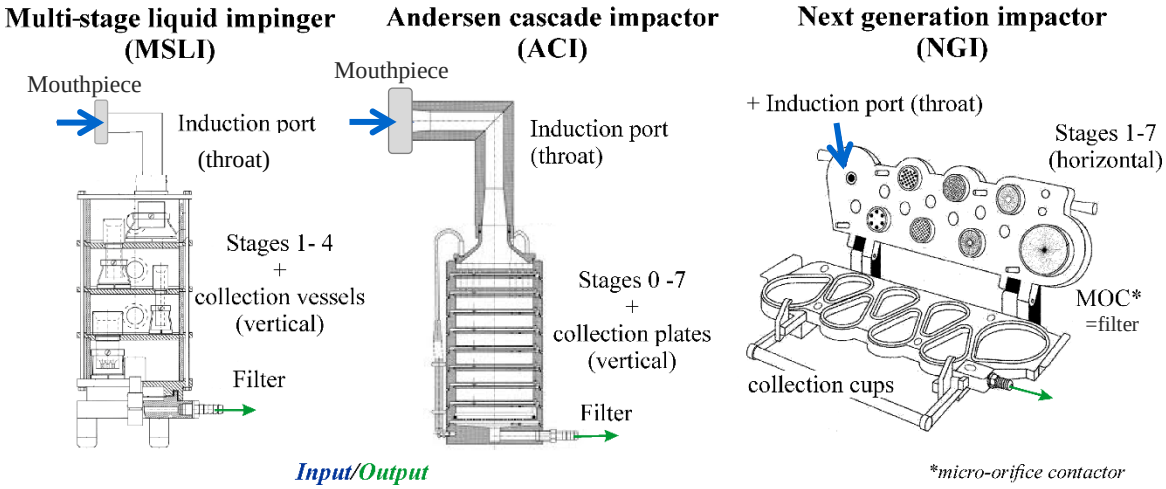


Figure 6: *The structure of the three impactor types most commonly used for aerodynamic size distribution and deposition assessments (Eur. Ph. Online 9.6).*

The most widely used devices for DPI testing are the ACI and the NGI. There are many structural differences between these three impactors, but studies showed that the comparison of aerodynamic measurements is quite the same with different devices and drugs also (*Taki et al., 2010*; *Yoshida et al., 2016*). As the presented study was measured with ACI, this will be discussed in detail. For the DPI measurements the Pharmacopeia recommends the use of 30 L/min (28.3 L/min) inhalation flow rate or pressure drop of 4 kPa (*Taki et al., 2010*). For the DPI measurements a pre-separator can be used equipped before the stages to collect the larger carrier particles during inhalation, especially if carrier-based DPIs are measured (to collect the separated 50-200 µm carriers). The collection plates of the ACI should be covered with some surfactants to avoid particles

sweeping away, thus making the parallel measurements more reproducible. On the lowest filter stage must be used a filter which can collect particles under 1 μm and protects the submicron particles pollute the measuring room.

2.2.2.2. In silico aerodynamic modelling

The *in silico* simulations can be performed by the latest version of the Stochastic Lung Deposition Model, which is a whole-lung deposition model originally developed by Koblinger and Hofmann (***Koblinger et al., 1985***; ***Hofmann et al., 2006***). In the Stochastic Lung Deposition Model, the particles are tracked from their inhalation until they deposit in the airways or leave the airways by exhalation. The model computes the fraction of the particles deposited in each anatomical region of the respiratory system (extrathoracic, bronchial and acinar) and the exhaled fraction, too (***Farkas et al., 2017***). The diversity of airway anatomical characteristics used in the modelling (airway lengths and diameters, branching and gravity angles) is described mathematically by probability density- and correlation functions. The input parameters of the deposition model include the inhalable particles properties and the breathing characteristics of the patient (***Table III***) (***Farkas et al., 2015***).

Table III: *Primary inputs of the in silico deposition model (table from* ***Farkas et al., 2015***).

Breathing parameters	**Aerosol parameters**
functional residual capacity	particle density
inhaled volume	size distribution type (monodisperse or polydisperse)
inhalation time	particle size (if monodisperse, or MMAD and GSD if polydisperse)
breath-hold time	number of size intervals (if polydisperse)
exhalation time	weights for each interval (if polydisperse)
breathing mode (mouth/nose)	particle shape (spherical or not)
particle inhalation mode (uniform/bolus)	shape factor (if not spherical)

2.2.2.3. Combination of the in vitro and in silico modelling

Application of both modelling allows for a more exact characterization of the aerodynamic properties of the samples and for specifying the correlation between the two procedures. Both the *in vitro* and *in silico* modelling have advantages (**Fig. 7**) and are useful for characterizing the aerodynamic behaviour of inhalable products. These techniques can be used as stand-alone methods but also to compensate for the disadvantages of the other method. *In vitro* measurements may provide input data (size distributions, MMAD, GSD, emitted dose) numerical modelling of airway deposition distribution of the inhaled aerosol drugs.

The breath-hold time after the inhalation of the drug, which is an input parameter of the deposition model, is an important factor influencing the amount of drug depositing in the airways (***Farkas et al., 2017***). It is clearly demonstrated that the length of the breath-hold after inhalation has significant impact on the deposited fraction of the particles (***Horváth et al., 2017***). However, this parameter is not taken into consideration during *in vitro* deposition measurements. By combining *in vitro* and *in silico* techniques the effect of this parameter can be accounted for. As mentioned before, the outputs of the *in silico* model is the fractions of drug doses deposited in the whole respiratory tract and in distinct regions of the airways (extrathoracic, lung and exhaled value) (***Kerekes et al., 2013***). Airway deposition of several commercially available DPIs and pMDIs were simulated by the Stochastic Lung Deposition Model (***Jókay et al., 2015***; ***Jókay et al., 2016***; ***Farkas et al., 2015***), which was previously validated against *in vivo* deposition data available in the open literature.

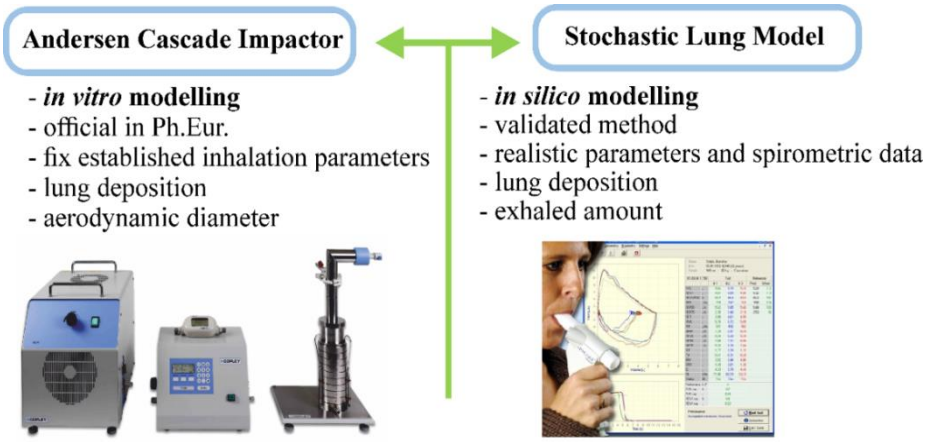


Figure 7*: Stochastic Lung Model advantages compared to in vitro aerodynamic testing (ACI) (****Chvatal et al., 2017****).*

3. AIM OF THE WORK

The aim of this PhD work was to prepare and characterise innovative **carrier-free DPI formulations** designed for local pulmonary drug delivery. In our institute, previous work on inhalable meloxicam (MX) microcomposite preparation by the pre-suspension spray drying method was reported (***Pomázi et al., 2011***; ***Pomázi et al., 2013***). The prepared inhalable particles have good aerodynamic properties for a possible local treatment of COPD or other lung inflammatory diseases.

- Therefore we aimed to evaluate a new **"spray drying from solution" technology**, where MX is dissolved in aqueous solution, without using any organic or health harming solvents. This innovative solution formulation was achieved with two technically feasible strategies:
    - first, with the use of the newly patented (*nr.: WO2006064298A1*) water-soluble salt form of MX, meloxicam potassium (**MXP**),
    - second, with increase of the water solubility of **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), which is easily scalable, controllable and preferred by the industry:
    - the spray drying properties were developed for two types of spray drying devices (Büchi B-191 and Büchi B-290) for the two different types of formulation techniques.
- We aimed to evaluate **carrier-free DPI formulations** with two different spray drying technologies and we followed the most important aspects and innovations for valuable results (***Fig. 8***):
    - 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.
    - For both formulations the **appropriate excipients** were used to enhance aerosolization or to create a special structure of the particles.

- **A comparison study** was made regarding spray drying yield, physicochemical properties (e.g. crystallinity, morphology, density) and aerodynamic behaviour to establish the advantages and disadvantages of the two formulation methods.
- The **aerodynamic behaviour** of the formulations was established with a combination of *in vitro-in silico* evaluation after 1 and 10 weeks of storage:
    - 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***), while
    - 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.

Both MXP and MX were used as model active ingredients for the production of inhalable carrier-free DPI particles. The formulation and analysis protocol was planned with the consideration of the FDA process development requirements (***FDA guidance, 2018***).

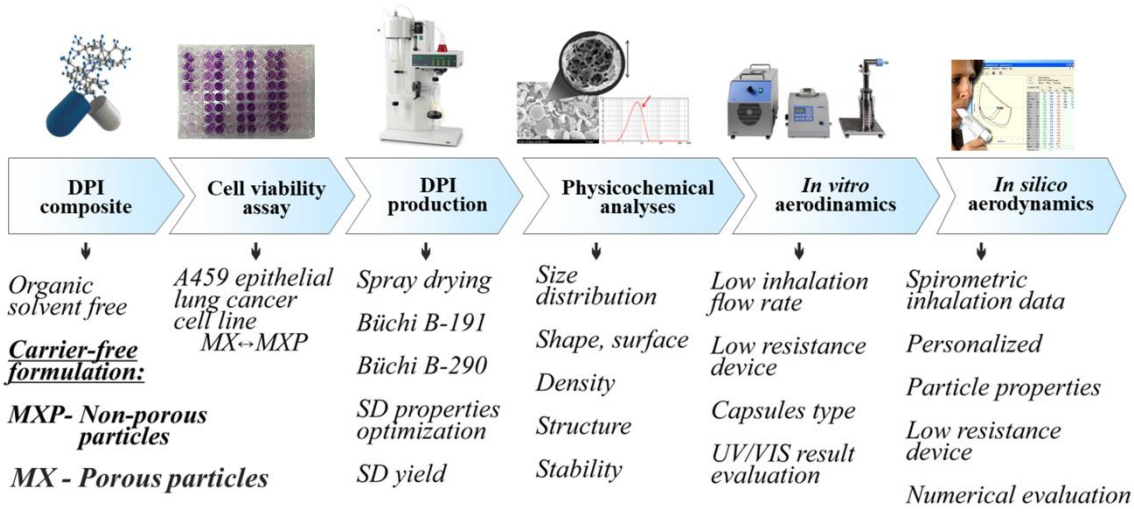


Figure 8*: The formulation strategy and analysis protocol of the planned PhD work. LPP=large porous particle formulation.*

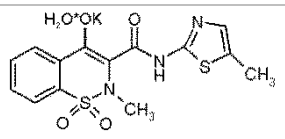
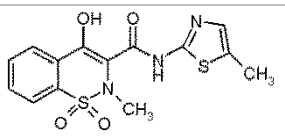
4. MATERIALS AND METHODS

4.1. Materials

4.1.1. Active pharmaceutical ingredients

MX inhibits the COX-2 enzyme and thus has anti-inflammatory effects (***Luger et al., 1996***) (provided by Egis Pharmaceuticals PLC., Budapest, Hungary). The low water soluble MX is usually processed in suspension forms if no organic solvent is required (micro or nano suspensions) (***Iurian et al., 2017***; ***Bartos et al., 2015***; ***Pomázi et al., 2013***) In previous studies it was demonstrated that low water-soluble MX can be administered in pulmonary application for the local treatment of pulmonary fibrosis, COPD and non-small cell lung cancer (***Szabó-Révész, 2018***; ***Tsubouchi et al., 2000***; ***Arafa et al., 2007***; ***Pomázi et al., 2014***). However, NSAIDs tend to cause allergic reaction, it was demonstrated that meloxicam can be used as a safe alternative for aspirin or NSAID hypersensitive patients also (***Bavek et al., 2007***). The other used API was the MXP, which has not been studied before, but it is a valuable water-soluble intermediate obtained from the synthesis of high-purity meloxicam (>99.90%) (***Mezei et al., 2009***). According to our aims, the meloxicam containing "spray drying from solution" DPI preparation method was achieved with two ways: by using the newly patented (*nr.: WO2006064298A1*) water-soluble MXP and by increasing the water solubility of MX with pH shift (***Mezei et al., 2012***). A preformulation study was made in order to establish the possible ways of increasing the MXP and MX concentrations in aqueous solutions (***Table IV***).

Table IV*: Solubility (mg/mL) of MXP and MX tested in solutions of different pH and temperature (**Horváth et al., 2016**).*

 **Meloxicam potassium (MXP)**		 **Meloxicam (MX)**	
Tested solvent (pH 7.0)	***S (mg/mL)***	***Tested solvent (37 °C)***	***S (mg/mL)***
25 °C	8.3	7.0	0.04
37 °C	13.1	7.4	0.93
80 °C	32.0	7.7	1.74
Temperature increase used for higher concentrations.		*pH shift used for higher concentrations.*	

4.1.2. Excipients

We chose the excipients according to the most important requirements of DPI formulations (such as morphology, size, surface area and hygroscopicity of the particles) to obtain good aerodynamic properties both for the non-porous and large porous particles (***Telko et al., 2005***). In all formulations L-leucine (LEU) (Sigma-Aldrich, USA) was used to decrease the aggregation between the particles and to protect the particles form moisture (***Rowe et al., 2009***). Besides, polyvinyl alcohol (PVA) (ISP Customer Service GmBH, Germany) and sodium hyaluronate (HA) (Acros Organics, Belgium) was used as a stabilizing agent in some formulations (***Rowe et al., 2009***). HA also functions as an absorption promoter (***Rowe et al., 2009***) and increases the viscosity of the primary solution to obtain large droplets and larger sized particles in porous formulations. For the porous formulation ammonium bicarbonate (AB) (Sigma-Aldrich, USA) was added as a progeny agent to promote low density and hollow or porous structure (***Cruz et al., 2011***).

4.2. Spray drying method for carrier-free DPI production

4.2.1. Sample preparation methods and spray drying

According to our aims, two types of carrier-free DPI formulations (non-porous and porous formulations (LPP technology)) were achieved with two different active ingredients (MXP and MX) to evaluate a “spray drying from solution” technology.

4.2.1.1. Non-porous formulations

The appropriate water solubility of MXP allows the using of a simplified, one-step particle engineering procedure for preparing the DPI formulation. The increasing temperature increases the water solubility of MXP, which is 8.3 mg/mL at room temperature (25 °C), 13.1 mg/mL at 37 °C and 32.0 mg/mL at 80 °C (at pH 7.0). Therefore, we used 70±5 °C purified water as a solvent, mixing with heated water for 10 minutes (400 rpm stirring). After cooling at 50 °C (max. 30 min, 400 rpm) without the recrystallization of MXP, solutions were mixed with the appropriate combination of LEU and PVA (***Table V***). PVA solutions were also prepared with heating one day before the spray drying. A magnetic stirrer with heating function was used for solvent homogenization (AREC.X heating magnetic stirrer, Velp Scientifica Srl, Italy).

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

	Composition of microparticles/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

4.2.1.2. Porous formulations

MX exhibits pH-dependent solubility: in buffers at pH 7.4–7.7 MX demonstrates solubility almost 43-fold higher (1.74±0.2 mg/mL, 37 °C, pH 7.7) than in distilled water (0.04±0.01 mg/mL, 37 °C, pH 7.0) (***Horváth et al., 2016***). Due to this characteristic, MX was dissolved at laboratory temperature (20±2 °C) at higher pH and stabilized with 1 M sodium hydroxide aqueous solution to prepare the stock solution for the spray drying (***Table V***). First, MX was dissolved in the high pH water (8.0±0.1 pH, stirring for 2 hours, 600 rpm) at 1.5 mg/mL constantly in each formulation. Secondly, 0.75 mg/mL LEU was dissolved (15 min, 600 rpm) in the MX solutions. Finally, HA was added to yield 0.15 and 0.30 mg/mL final concentrations. The appropriate concentration HA solutions were prepared the day before the spray drying (stirring for 24 hours at 400 rpm). In order to formulate porous particles, different concentrations of AB were added, which functions as a pouring agent and facilitates porous structure preparation. AB in concentrations of 1.5 and 2.0 mg/mL was dissolved maximum 5 min before the spray drying (stirring for 2 min, 200 rpm) to minimize its decomposition into CO_2 and NH_3 before spray drying.

4.2.2. Spray drying process parameters and drying efficacy

Our aim was to prepare non-porous and porous formulations containing MX and MXP with different process parameters of "spray drying from solution" technique (***Table VI***). 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. The used AB amount was not taken in consideration as it decomposes during drying. The parameters presented in ***table VI*** were chosen to reach a yield above 55% at each sample production. Each formulation was spray dried in triplicate.

Table VI: *The spray drying process parameters chosen for the two types of carrier-free DPI preparation.*

	Non-porous formulations	**Porous formulations**
Active ingredient	MXP	MX
Feed stock	solution	solution
Spray drier type	Büchi B-191	Büchi B-290
Inlet temperature	140 °C	200 °C
Outlet temperature	75-80 °C	95-100 °C
Aspirator rate	75%	100%
Gas flow rate	600 L/h	414 L/h
Feed pump	2.5 mL/min	9.0 mL/min

The actual API content (%) of the spray dried particles was measured by dissolving 1.0–1.1 mg product in 25 mL of methanol:7.4 pH phosphate buffer (60:40 w/w%), which solution was used for the aerodynamic assessment. too. The mother solutions were mixed for 10 min, 600 rpm and the API content was quantified by UV/Vis spectrophotometry (ATI-Unicam UV/VIS Spectrophotometer, UK) at a wavelength of 362 nm. Each sample was measured in triplicate.

4.3. Cell viability assay

Literature data report about the cytotoxicity assay of meloxicam-containing microcomposites, prepared with spray drying from suspension technology, on monolayers of Calu-3 cells (***Ambrus et al., 2011***). As no measurements with MXP had been published before, it was essential to establish the safety of the salt form as well as the base of MX.

The selected APIs were tested on human epithelial A549 lung carcinoma cells (ATCC®, USA) (***Fig. 9***). Cells were maintained in DMEM/F-12 50/50 nutrient mixture (Cellgro, USA) mixed with 10% foetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin in 5% carbon dioxide environment at 37 °C. The medium was changed every other day. MTT assay was carried out (***Mosmann, 1983***; ***Gerlier et al., 1986***). In brief, A549 cells were seeded in 96-well cell culture plates with lid (Corning™, NY) at a density of 10,000 cells/well, established with a haemocytometer (Fisher Scientific, PA). Cells were pre-incubated for 24 hours at 37 °C, in 5% carbon dioxide to assist cell attachment. The pure drugs were dispersed in DMEM/F-12 medium to obtain final active ingredient concentrations of 0.01, 0.1, 1, 2, 5 and 10 mg/mL (***Chvatal et al., 2018***). The cells were then exposed to varying concentrations of MX and MXP for 1 hour (37 °C). Negative controls were incubated in DMEM/F-12 medium and 100% DMSO (Fischer Scientific, PA) was used as a positive control. After 1 hour of incubation, cells were washed with DMEM/F-12 medium and then MTT solution was added to each well and incubated at 37 °C for 3 hours.

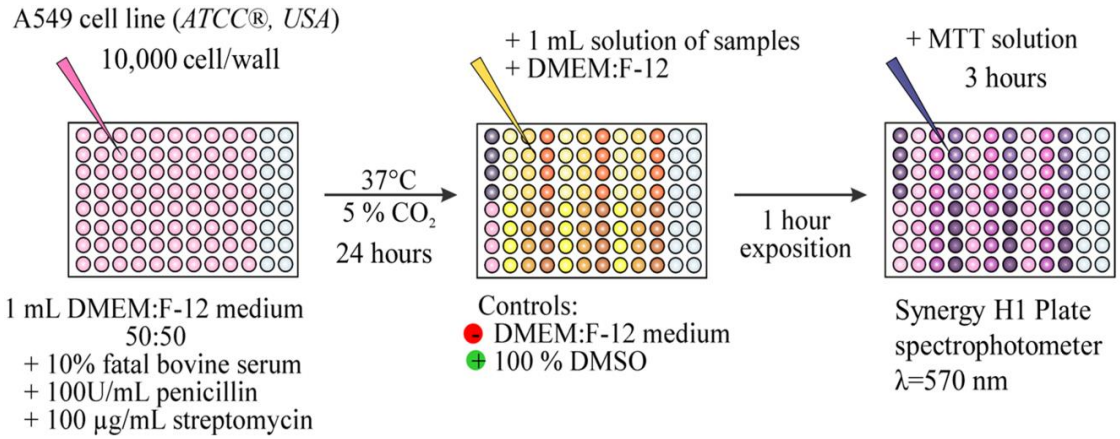


Figure 9: *Cell viability measurements (**Chvatal et al., 2017**)*

The formazan crystals formed by the viable cells were dissolved with 100% DMSO and the viable cells were measured *via* Synergy H[1]plate spectrophotometer (Biotek®, VT) at 570 nm. The absorbance reading of the blank was subtracted from all samples. Absorbance (*abs*) readings from test samples were then divided by those of the control (DMEM/F-12 medium) and multiplied by 100 to give percentage cell viability (**Eq. 1**). Absorbance values greater than the control indicate cell proliferation, while lower values

suggest cell death or inhibition of proliferation. All the measurements were made in triplicate.

$$\% \text{ viable cells} = \left(\frac{abs_{sample} - abs_{blank}}{abs_{control} - abs_{blank}}\right) \times 100 \quad \textbf{(Eq. 1)}$$

4.4. Structural analyses

4.4.1. Identification of active pharmaceutical ingredient

To investigate the identity of API in the formulations, Raman spectroscopy was applied using Thermo Fisher DXR Dispersive Raman with CCD camera (Thermo Fisher Sci. Inc., Waltham, USA). The following parameters were used during the measurements: laser diode operating at a wavelength of 780 nm; the applied laser power was 6-24 mW at 25 μm slit aperture size on a 2 μm spot size; spectra were collected with 6 sec exposure time of 20 scanning in the spectral range of 3300–200 cm^{-1}. As a reference, MX and MXP was dissolved in sodium hydroxide aqueous solution and purified water as well, with the same method for the spray drying, and recrystallized (at 40 °C, 24 hours) to see the changes while dissolving APIs (***Chvatal et al., 2019***).

4.4.2. Identification of the crystallinity of powders

To establish the crystalline or amorphous character of the spray dried samples, 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 the interval 3–40°. Measurement conditions were as follows: target, Cu; filter, Ni; voltage, 40 kV; current, 40 mA; time constant, 0.1 s; angular step 0.010°.

4.5. Morphology of the particles

Scanning electron microscopy (SEM) (Hitachi S4700, Hitachi Scientific Ltd., Japan) was used to characterize the morphology of the spray dried formulations, applying 10–15 kV high voltage set and 1.3–13.0 mPa air pressure. A high vacuum evaporator and argon atmosphere were used to sputter-coat the samples with gold-palladium in order to make them conductive (Bio-Rad SC 502, VG Microtech, UK).

4.6. Particle size analyses

The volume median diameter size 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) was determined by using laser diffraction (Mastersizer 2000 equipped with a Sirocco dry disperser, Malvern Instruments Ltd., UK). The dispersing pressure was 2 bars. The particle size distribution was characterized by the D[0.1] (10% of the volume distribution is below this value), D[0.5] and D[0.9] (90% of the volume distribution is below this value) values. The size distribution Span was calculated according to **Eq. 2**. Each sample was measured in triplicate.

$$Span = \frac{D[0.9] - D[0.1]}{D[0.5]} \quad \textbf{(Eq. 2)}$$

4.7. Density measurements

The density of the formulations was measured using a 5 mL cylinder, filled with 2–4 mL powder (for bulk density) and tapped 1000 times (for tap density) using a tapping apparatus Pharma test PT-TD1 (Pharma Test Apparatebau AG, Germany) (***Eur. Ph. Online 9.6, Chapter 2.9.34.***). All samples were measured in triplicate.

4.8. Aerodynamic characterisation

4.8.1. In vitro assessment

The aerosolization efficacy of the spray dried formulations was assessed *in vitro*, using an Apparatus D in European Pharmacopoeia (Andersen cascade impactor, Copley Scientific, Switzerland) with high-capacity pump (Model HCP5, Copley Scientific Ltd., UK) and critical flow controller (Model TPK, Copley Scientific Ltd., UK) (***Eur. Ph. Online 9.6., Chapter 2.9.18.***). The measurement properties are presented in ***Table VII***. The plates of the impactor were coated with 1% w/v mixture of Span 85 and cyclohexane to allow for the attachment of floating particles. On filter stage a 1.0 μm porosity glass filter was used (Type A/E, Pall Corporation, USA). Between two inhalation minimum 5 s break was allowing the particles to deposit with gravitation also. After two repetitions of 4 s actuation, the inhalation device, the capsules, the mouth piece (extra support to connect the device to the induction port), induction port, the collection plates and the filter were washed in separate vessel with methanol:7.4 pH phosphate buffer (60:40 v/v%) to collect

and dissolve the deposited API. The collected MX and MXP in each vessel were quantified by UV/Vis spectrophotometry (ATI-Unicam UV/VIS Spectrophotometer, UK) at a wavelength of 362 nm. Aerodynamic properties (FPF, MMAD and GSD) were calculated from a plot of the cumulative percentage undersize of the API on log probability scale against the effective cut-off diameter using KaleidaGraph program (***Colombo et al., 2013***) (***Fig. 5***). EF was expressed as percentage of the API remaining in the capsules and the device after inhalation divided by the total loaded API dose.

Table VII: *Andersen cascade impactor assessment parameters.*

Parameters/Properties	**Value/Type**
Inhalation flow rate	30±1 L/min
Inhalation time	4 s
Inhalation actuation	2 repetitions each capsule
Inhalation device	Breezhaler®, single dose
Capsules	size 3, transparent, HPMC (Capsugel)
Sample filling	2–2.5 mg of powder

4.8.2. In silico modelling

In order to simulate the realistic breathing modes of the patients, measured spirometry data of the individuals inhaling through Breezhaler® device were adopted in the Stochastic Lung Model Deposition 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: inhaled volume (IV) 1.7 L, inhalation time (t_{in}) 2.04 s, exhalation time (t_{ex}) 3.0 s, mean inhalation flow rate (Q) 50.1 L/min (***Chvatal et al., 2017***). ***Fig. 10*** shows the more realistic, time-dependent inhalation mean pattern implemented into the numerical model, which was used for the sample behaviour simulation. As the breath hold time has significant effect on the particle deposition we included it in the simulations $t_{b\text{-}h}$ = 5.0 s and 10.0 s (***Farkas et al., 2017***). The realistic *in silico* modelling gives the exact aerodynamic behaviour of the samples, completing the testing with extra data. Besides the extrathoracic and lung deposition doses, the exhaled amount can also be determined. The modelling was evaluated using the *in vitro* aerodynamic properties of 1 and 10 weeks of storage (***Chvatal et al., 2018***).

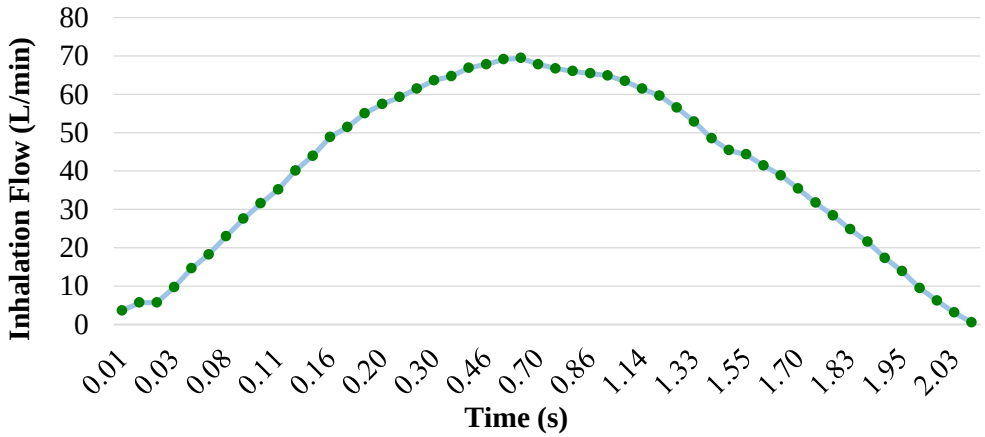


Figure 10**: General COPD inhalation profile through Breezhaler® using spirometry data by Chapman et al., 2011* (Chvatal et al., 2017***).

4.9. Stability measurements

The most important properties (size, density, aerodynamic properties) determining the aerodynamic 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. Results of the stability properties will be discussed in each chapter separately (size, density and aerodynamic characterisation).

4.10. Statistical analyses

Statistical analyses were taken using t-test calculation at 0.05 significance level and one-tailed hypothesis. T and p values were calculated from the tree parallel measurements of the compared results. Differences were considered significant if $p<0.05$ (***Social Science Statistics Online***).

5. RESULTS

5.1. Carrier-free DPI spray drying efficacy

Two different types of spray drying machines and methods were used to prepare MXP and MX containing carrier-free DPIs. Although the reference spray dried APIs (MXP-SD and MX-SD) were produced with 10 and 20% higher yield compared to the formulations, the use of excipient was essential for the optimal products. Both non-porous and porous formulations (LPP formulation type) were produced with an acceptable spray drying yield above 60%. However, the reference spray dried APIs had the highest spray drying yield (MXP-SD 72.0% and MX-SD 87.7%) the use of excipients was essential for the enhanced aerodynamic behaviour. 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. The difference between the theoretical and real (after spray drying) API contents were less than 10% (***Table VIII***).

Table VIII*: Spray dried yield and the final API content of the carrier-free DPI microcomposites (%). Data are presented as mean ± S.D., n=3. *Reference spray dried APIs.*

	Spray drying yield (%)	**Final API content (%)**
Non-porous formulations		
*MXP-SD**	*72.0±2.42*	*98.5±1.01*
MXP/LEU^{20}	62.0±1.94	50.3±0.24
MXP/LEU^{40}	60.0±2.37	33.3±0.28
MXP/LEU^{20}/PVA$^{2.5}$	60.5±2.48	38.3±2.68
MXP/LEU^{40}/PVA$^{2.0}$	63.7±2.95	32.3±0.32
Porous formulations		
*MX-SD**	*87.7±2.76*	*89.7±1.3*
MX/LEU/HA$^{0.30}$/AB$^{1.5}$	61.6±2.13	53.6±2.1
MX/LEU/HA$^{0.15}$/AB$^{1.5}$	70.0±2.39	55.3±0.3
MX/LEU/HA$^{0.30}$/AB$^{2.0}$	62.3±2.07	50.7±1.0
MX/LEU/HA$^{0.15}$/AB$^{2.0}$	67.5±2.75	58.7±1.6

5.2. Cell viability assays

In this MTT study, the cellular metabolic activity as a proxy for cell viability of MXP and MX were compared on human epithelial A549 lung carcinoma cells. Viable cells (containing NAD(P)H-dependent oxidoreductase enzymes) can reduce the MTT reagent to formazan crystals which have purple colour. The more viable cells can be found in a plate the higher absorbance is measured at 570 nm wavelength (darker solution=greater metabolically activity). The cytotoxicity of pure drug samples was compared with negative and positive controls, medium and DMSO respectively. In the presence of DMSO, only 10% cell viability was observed. MX, MXP and formulations of MXP exhibited high cytotoxic effect at higher concentrations of 1, 2, 5 and 10 mg/L compared to the negative control (***Chvatal et al., 2018***). No difference in cytotoxicity was observed in the case of the MXP and MX forms at 0.1 and 0.01 mg/mL concentrations, as the solubility of the two forms are almost the same on the measuring conditions (MX: 0.933±0.054 mg/mL, while MXP: 0.729±0.001 mg/mL, measured at 37 °C, in 7.4 pH buffer) (***Horváth et al., 2016***) (***Fig. 11***). It was clarified that MXP has a similar cytotoxic effect on A549 cells as MX and both can be safely used at 0.1 and 0.01 mg/mL concentrations.

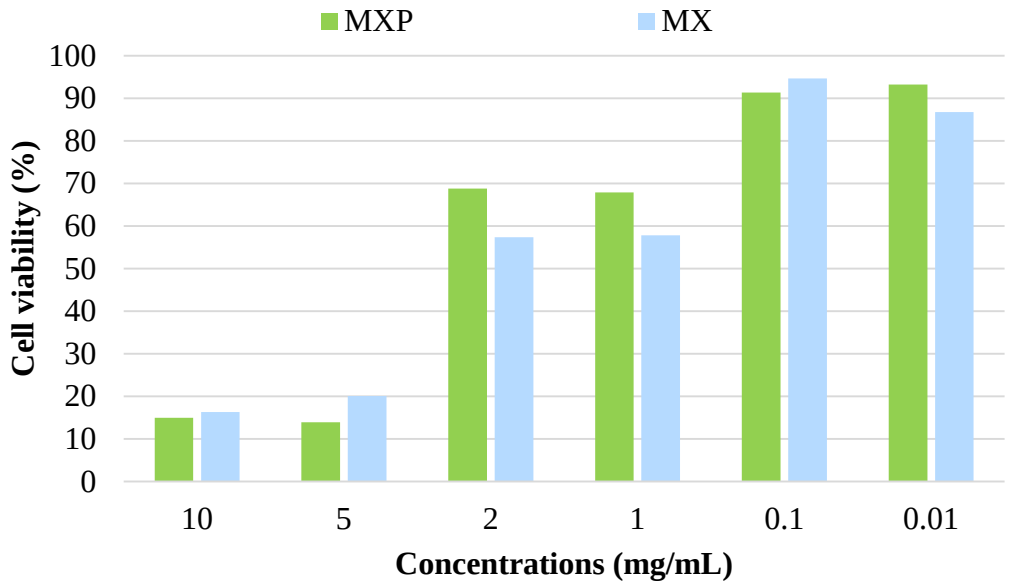


Figure 11: *Cytotoxicity of raw MX and MXP (Chvatal et al., 2017). Data are presented as mean (n=3), S.D. was less than 0.1% for each concentration.*

5.3. Structural analyses of the carrier-free DPIs

5.3.1. Identification of APIs

The Raman spectra of raw MX (MX-raw) show characteristic bands at 1155, 1309, 1540 and 1595 cm^{-1} (***Fig. 12***) (***Bio-Rad Laboratories, Inc. SpectraBase; Meloxicam***). These Raman spectra are similar to the spectra of MX sodium salt according to the Bio-Rad Laboratories database (***Bio-Rad Laboratories, Inc. SpectraBase; Meloxicam sodium***). Based on this result, it was established that during the dissolution of MX (in pH 8.0±0.1 sodium hydroxide aqueous solution) *in situ* forming of MX sodium salt occurred. Spray dried MX (MX-SD) and formulations with excipients exhibit the same spectra as MX sodium salt with characteristic Raman bands at 1390 and 1595 cm^{-1}, indicating MX sodium salt form is present in spray dried formulations (***Chvatal et al., 2019***).

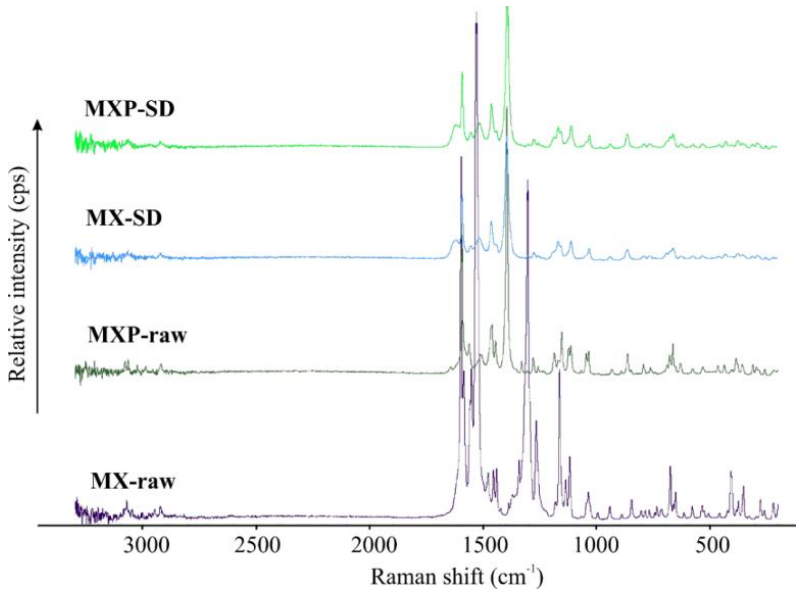


Figure 12*: Raman spectra of the spray dried (-SD) and raw (-raw) active ingredients.*

5.3.2. Crystal structure

X-Ray powder diffraction was used to characterize the crystalline state of MXP and MX after the spray drying process. 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) (***Aytekin et al., 2018***; ***Chvatal et al., 2019***). The fact that the characteristic peaks of crystalline APIs are missing from the diffractogram of spray dried samples (MXP-SD, MX-SD) indicates that the raw material becomes amorphous during the spray drying (***Fig. 13***). Characteristic

peak of MXP at 6.04° 2-theta appears in the non-porous formulations, indicating a low level of crystallinity. The same semi crystal form can be observed in the case of porous formulations: lower intensity peaks at 6.6°, 11.4°, 13°, 13.6° and 19.2° 2-theta. The semi crystal form was observed in the presence of LEU. We detected the same characteristic peaks of LEU that literature data reported (***Najafabadi et al., 2004***; ***Li et al., 20016***; ***Chvatal et al., 2017***). The characteristic peak 5.9° 2-theta of LEU could be observed on the spectra of the formulations but it was broader and with much lower intensity. These low intensity peaks demonstrate the low crystallinity of LEU after spray drying. The presence of PVA and HA has no effect on the diffractogram of the samples.

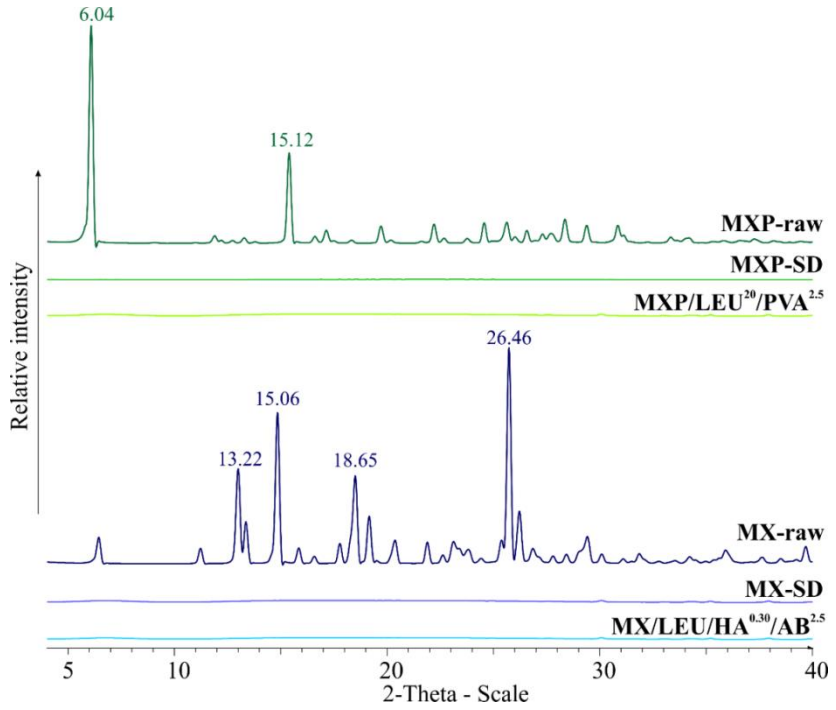


Figure 13: *XRPD spectra of the raw (-raw) and spray dried (-SD) APIs and one of each types of formulation.*

5.4. Morphology

Raw MXP and MX were characterized by large angular crystals unsuitable for pulmonary application (***Fig. 14***). For the spray dried samples (MXP-SD and MX-SD) particle size was dramatically reduced to less than 10 μm, reaching the inhalable size range (***Chvatal et al., 2015***). Regarding morphology, these particles were found to be spherical (***Fig. 14***). The SEM pictures (***Fig. 14***) revealed that the excipient-free spray dried sample (MX-SD and MXP-SD) had a slightly rough surface and an almost spherical shape for both APIs.

In the case of spray drying, APIs enrich on the surface of the droplet during the solvent evaporation phase. The formed fixed crust cannot follow the quick form changes during evaporation and slightly sinks in wrinkled particles.

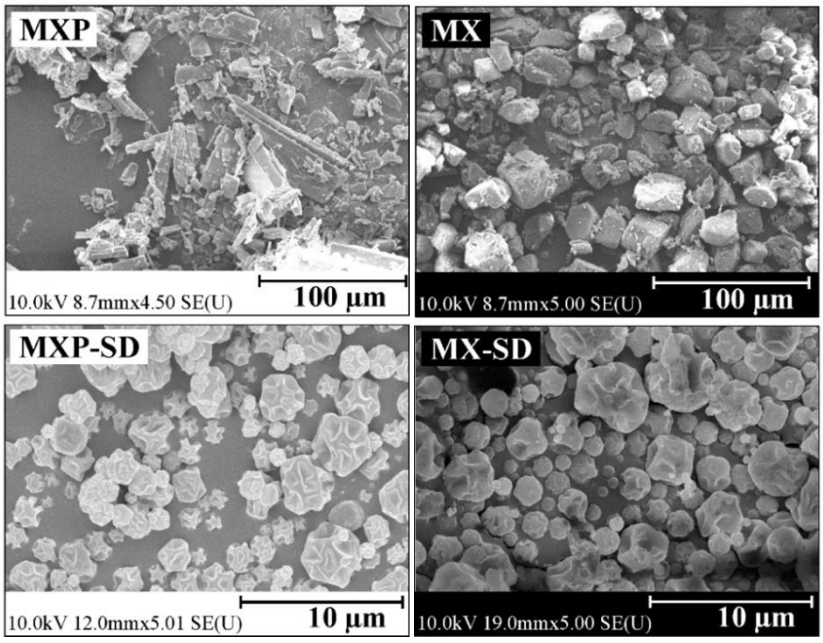


***Figure 14**: Electron microscopy pictures of the raw and spray dried (-SD) active ingredients. Pictures present the spherical, slightly rough surface particles that were formed form during spray drying thanks to the characteristics of the MXP and MX.*

The addition of LEU (in the used concentrations: 0.5:1 LEU:MX; 1:1 and 2:1 LEU:MXP) shifts the spherical morphology to a shell-formed appearance as supported (***Vehring et al., 2008***). The low diffusion coefficient of LEU (*Pe*>1) increases particle enrichment in the droplet surface during spray drying. The quick drying core crumples and forms a rough surface (***Chvatal et al., 2019***). The surface accumulation and surface modifying of LEU had minimized contact area thus can reduce the adhesion between particles. It also could reduce the attachment to capsule or inhalation device wall during inhalation which may result in higher emitted fraction (***Aquino et al, 2012***; ***Mangal et al., 2015***; ***Chew et al., 2005***). PVA was used in the non-porous formulations to reduce the cohesion between particles and to stabilize the formed morphology (***Chvatal et al., 2015***). Using the appropriate amount of PVA decreased the aggregation and uniform structured particles could be detected between the (***Fig. 15***). Its individual particles are assumed to have a monodisperse size distribution and better aerodynamic properties.

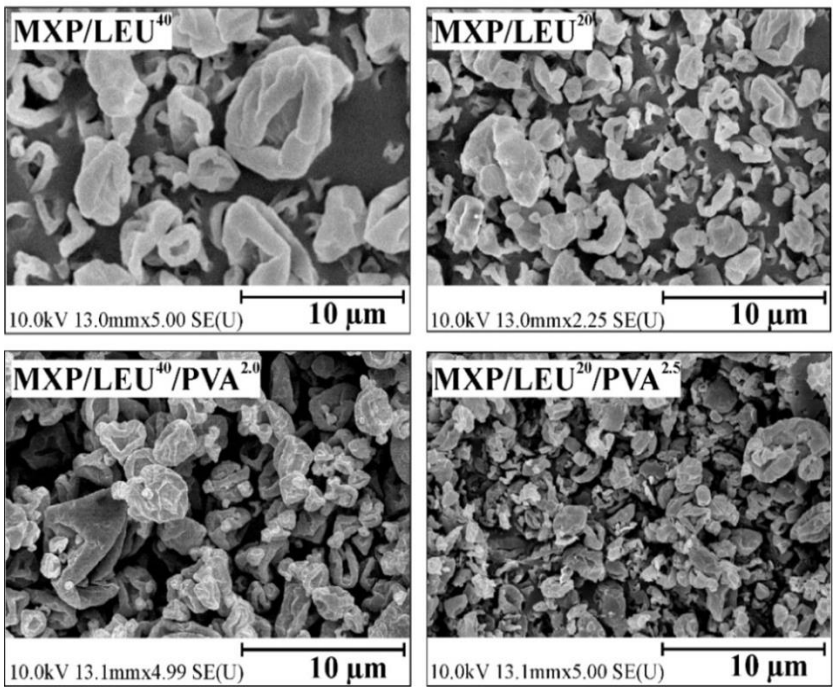


Figure 15*: Electron microscopy pictures of the MXP non-porous formulations presenting the shell-form morphology caused by the LEU content.*

HA, as well as PVA, was used in order to stabilize the structure of formulations *(**Martinelli et al., 2017**)*. However, literature data report that sodium hyaluronate could promote surface roughness of spray dried particles (***Li et al., 2017***), we did not detected the same intensive effect (***Chvatal et al., 2019***). However the surface roughness of porous formulations was much lower, slight wrinkles could be detected on the surface of the large sized spherical particles. These porous particles exhibit significantly different morphology than non-porous formulations in which AB was the main role. The SEM pictures also demonstrated the porogen effect of AB, which is well described in literature (***Cruz et al., 2011***; ***Nolan et al., 2009***; ***Gervelas et al., 2007***). The change in shape could arise from the gas formation from AB during the drying procedure, which "blows" up the structure and forms the presented larger sized spheres (***Fig. 16***). Other evidence of the porous structure can be detected in the broken shells, which demonstrate the internal hollow structure and low density of porous formulations. Between these large sized spheres the cohesion forces could be also lowered and high dispersity powders were formed (***Fig. 16***).

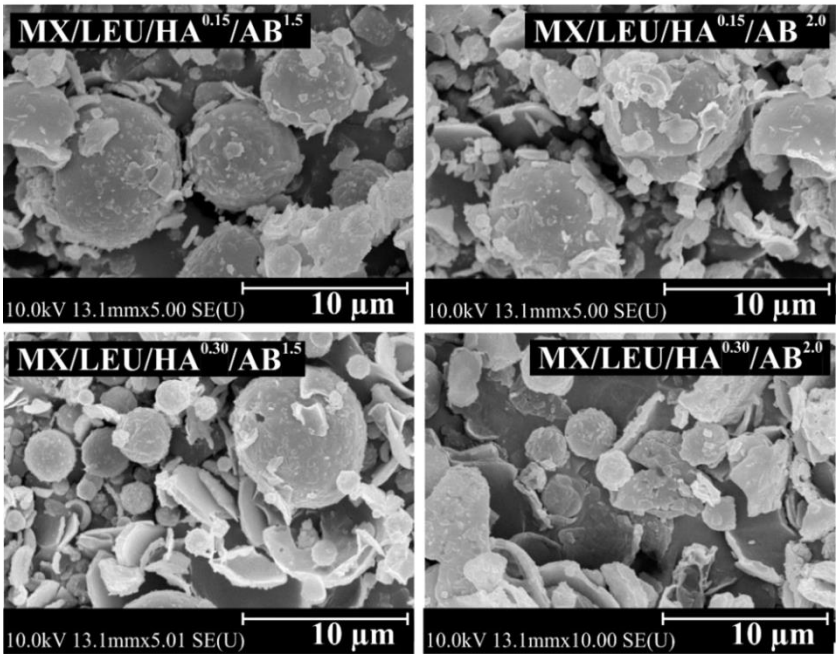


Figure 16*: Electron microscopy pictures of the MX porous particle formulations presenting the large spheres with internal hollow structure.*

5.5. Particle size analyses

Despite the fact that each formulation type was prepared using similar drying parameters, the final particle size distributions were different (***Table IX***). The medium geometric diameter (D[0.5]) of the reference spray dried particles MXP-SD and MX-SD was around 3.0–3.2 µm. The Span value was around 1.4 which refers to the narrow size distribution. In case of non-porous formulations 20 mg/mL LEU concentration had no effect on the size, the same geometric diameter was observed as the reference spray dried MXP (***Table IX***). With higher amount of LEU a slight size increasing was detected (with 20 mg/mL LEU 3.06 µm, while 40 mg/mL LEU shows 3.62 µm), but in non-porous formulations together with PVA this size increase effect was not significant. Similar conclusions reported as other studies, that incorporating LEU in the formulation is not increasing the geometric diameter (***Pham et al., 2013***; ***Aquino et al., 2012***). The PVA concentrations had no significant effect on the size distributions of particles (***Chvatal et al., 2015***). All the non-porous formulations had geometric diameter in the aimed 1-5 µm size range and have narrow size distributions (Span ≤1.8).

In the case of porous formulation the aimed larger geometric diameter (larger than 4.9 µm up to 5.7 µm) could be achieved. Geometric diameter was increased significantly with the higher HA concertation (from 4.9 µm to 5.7 µm with 0.15 and 0.30 mg/mL HA

concentrations respectively). This increase probably arises from an increase of the viscosity of the spray dried solution leading to larger droplets and thus resulting in larger particle size (***Li et al, 2017***). Geometric diameter larger than 5 μm was caused by the combination of HA (increases the viscosity of the spray dried solution, leading to larger droplets) and of AB (forms pores as AB decomposes into water and gas during the drying process, thus increases the size of particles) (***Cruz et al., 2011***; ***Pham et al., 2015***). Formulations prepared with 1.5 and 2.0 mg/mL AB concentrations had the same geometric diameter (***Chvatal et al., 2019***). Porous formulations also have narrow size distribution with Span ≤2.0. No significant differences were detected in the geometric diameter and the size distribution of the particles measured at 1st week and 10 weeks after of storage.

Table IX: *Size distribution of the carrier-free DPI samples. Data are presented as mean ± S.D., n=3. *Reference spray dried API.*

	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

5.6. Density measurements

When non-porous and porous particles were compared, it was established that there are significant differences in the tap density of the two formulation types. ***Fig. 17*** clearly shows that the porous formulations had lower tap density (<0.17 g/cm^3) than the non-porous formulations (>0.30 g/cm^3). The density of the reference spray dried APIs (MXP-

SD and MX-SD) was around 0.37 g/cm^3. The same tap density was observed in the case of non-porous formulations. In the used concentrations LEU content had no significant effect on the densities of both formulations. This observation was similar to other studies (***Aquino et al., 2012***). Non-porous formulations with PVA+LEU combination decreased the density from 0.37 till 0.34 g/cm^3 (MXP/LEU^{20}/PVA$^{0.25}$ is the lowest), but the difference was not significant. Despite the fact that polymers increase the viscosity of solutions, PVA and HA had no significant effect on the density of the spray dried particles (***Chvatal et al., 2017***; ***Chvatal et al., 2019***). There were detected significant differences in the tap densities of the two formulation types. The low density of porous formulations (0.09–0.16 g/cm^3) was in connection with the AB content, which forms the porous structure of the powders due to its bulking properties. Despite the large geometric diameter (≥5 µm), porous formulations exhibited the lowest tap density which may offer better flowability properties. The 2.0 and 1.5 mg/mL AB contents had almost the same density decreasing effect. The prepared porous formulations reached the aimed tap density of <0.20 g/cm^3 (the lowest was 0.09 g/cm^3 for MX/LEU/HA$^{0.3}$/AB^2).

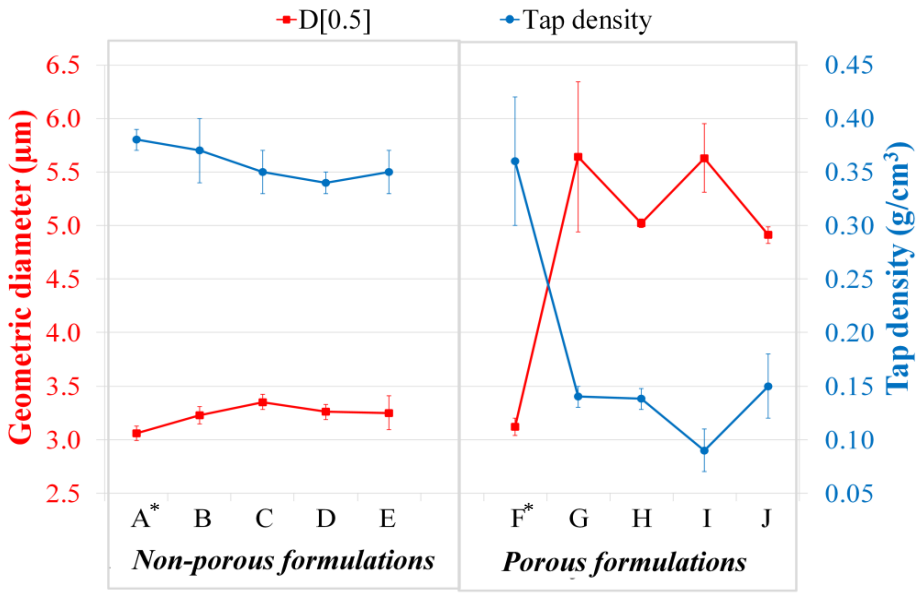


Figure 17: *Comparison of the median geometric diameter (expressed as D[0.5]) and tap density of the formulations (B: MXP/LEU^{20}, C: MXP/LEU^{40}, D: MXP/LEU^{20}/PVA$^{2.5}$, E: MXP/LEU^{40}/PVA2, G: MX/LEU/HA$^{0.3}$/AB$^{1.5}$, H: MX/LEU/HA$^{0.15}$/AB$^{1.5}$, I: MX/LEU/HA$^{0.3}$/AB^2 and J: MX/LEU/HA$^{0.15}$/AB^2). *Reference spray dried APIs (A: MXP-SD and F: MX-SD). Data are represented as mean ± S.D., n=3.*

5.7. Aerodynamic characterisations

5.7.1. In vitro assessment

The aerodynamic properties of powders were tested at 30 L/min, simulating low inhalation flow rate in Andersen cascade impactor. 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. Both non-porous and porous formulations were considered stable under the tested conditions (stored in desiccator, at 23±1 °C).

Briefly, the aerodynamic properties of the reference spray dried MXP and MX (with no excipients) were not increasing the *in vitro* aerodynamic properties of the commercially available DPIs and may not be efficient for pulmonary treatment (EF ≤59.1% and FPF ≤38.6%) (***Table X***). The low EF and FPF indicates that almost 40–50% of the drug remained in the capsules or device, while approximatively 60–70% of the loaded drug could not reach the site of action in the lungs.

Table X: *Aerodynamic properties tested at 30 L/min inhalation flow rate in Andersen cascade impactor. EF = emitted fraction, FPF = fine particle fraction. Data are represented as mean ±S.D., n=3. *Reference spray dried APIs.*

	EF (%)		**FPF (%)**	
	1st week	***10th week***	***1st week***	***10th week***
Non-porous formulations				
*MXP-SD**	*61.3±2.8*	*59.4±1.4*	*32.3±2.5*	*31.6±3.5*
MXP/LEU^{20}	72.7±5.7	77.8±2.4	48.6±7.0	52.8±0.8
MXP/LEU^{40}	69.1±1.5	68.8±4.2	42.9±1.2	46.3±5.9
$MXP/LEU^{20}/PVA^{2.5}$	77.0±3.2	76.6±3.3	50.9±1.9	50.3±6.1
$MXP/LEU^{40}/PVA^{2.0}$	67.5±5.6	71.2±4.1	50.5±2.9	50.9±1.9
Porous formulations				
*MX-SD**	*53.6±10.5*	*50.4±6.7*	*38.6±4.7*	*37.9±4.8*
$MX/LEU/HA^{0.30}/AB^{1.5}$	79.5±4.2	79.4±2.3	54.5±2.1	55.9±2.3
$MX/LEU/HA^{0.15}/AB^{1.5}$	77.5±6.8	76.1±9.9	57.4±5.6	54.5±10.1
$MX/LEU/HA^{0.30}/AB^{2.0}$	79.5±5.5	85.4±3.2	59.7±4.0	60.5±1.6
$MX/LEU/HA^{0.15}/AB^{2.0}$	82.8±6.8	82.3±8.3	65.8±3.2	63.0±4.5

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, the different LEU concentration had no significant effect on the FPF comparing (***Table X***). The additional excipient

concentrations increased the aerosolization of both formulations by increasing dispersity, modifying morphology and reducing density of particles. We detected significant differences between the EF (non-porous formulations 67–77% and Porous formulations 76–85%) and FPF (non-porous formulations 42–52% and Porous formulations 54–65%) of the two types of formulations. It can be demonstrated, that the used AB concentrations had relevant effect on the aerodynamic behaviour of porous formulations. Porous particles had significantly lower tap density (comparing to non-porous particles) which resulted improved lung deposition (FPF 54.5–65.8%). Other studies reported the same correlation between density and aerodynamics: the lower the tap density (0.04–0.25 g/cm^3), the higher the FPF is (***Bosquillon et al., 2001***). Porous particles prepared with 1.5 mg/mL AB concentrations had lower FPF (≤57.4%) than formulations containing 2.0 mg/mL AB (≥59.7%) (***Fig. 18***). The increased AB concentration (2.0 mg/mL) resulted in the highest FPF with 65.8% 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 contents had no significant effect on the EF or FPF (***Chvatal et al., 2019***).

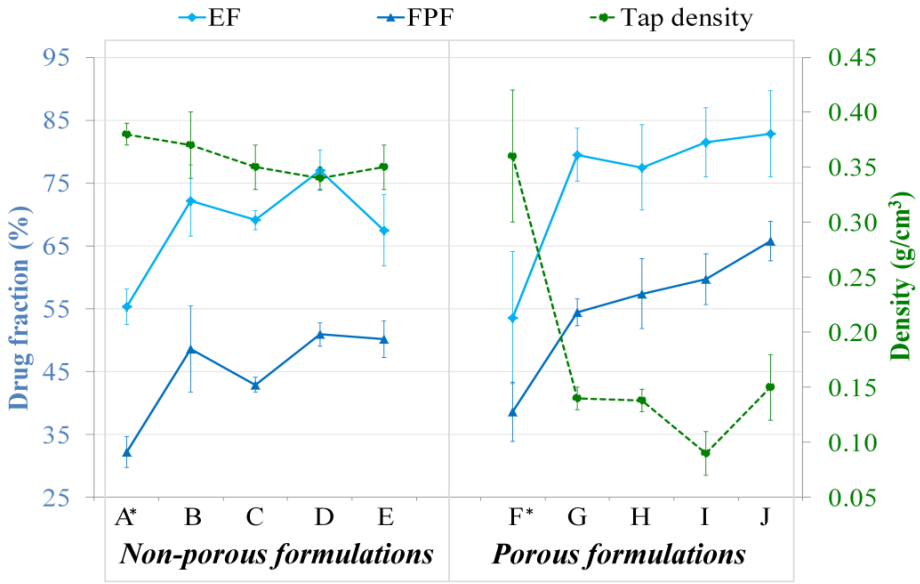


Figure 18: *Comparison of the fine particle fraction (FPF) and emitted fraction (EF) with the tap density of non-porous (B: MXP/LEU^{20}, C: MXP/LEU^{40}, D: MXP/LEU^{20}/PVA$^{2.5}$, E: MXP/LEU^{40}/PVA^2) and porous formulations (G: MX/LEU/HA$^{0.3}$/AB$^{1.5}$, H: MX/LEU/HA$^{0.15}$/AB$^{1.5}$, I: MX/LEU/HA$^{0.3}$/AB^2 and J: MX/LEU/HA$^{0.15}$/AB^2). *Reference spray dried particles (A: MXP-SD and F: MX-SD). Data are represented as mean ± S.D., n=3.*

The presented low density porous formulations (<0.17 g/cm^3) had better aerosolization properties and could reach the lower airways more easily (EF≥76.1% and FPF≥54.5%) than the smaller but denser non-porous particles (EF≤62.1% and FPF≤41.2%). Beside EF and FPF, MMAD is another important *in vitro* value describing the real aerodynamic diameter during inhalation. The criteria of the inhalation products are to reach 1-5 μm aerodynamic diameter which offers effective lung deposition *via* impaction (***chapter 2.2.1***.). The MMAD of the particles are mostly influenced of the geometric diameter of the particles (if is a dense structure), but the morphology and density properties are also effecting. The presented porous formulations and non-porous particles resulted in the same MMAD values (average 2.6 μm), despite of their significantly different geometric diameter, which is in connection with the density properties (***Table XI***). As an additional value the GSD was also determined: this value expresses the aerodynamic distribution of the particles during inhalation. GSD implies a lower particle size tail in the distribution while higher value implies non homogenous distribution in the lungs (***Musante et al., 2002***). Non-porous formulations have lower GSD around 1.5 while porous formulations around 1.8 which can be considered narrow distributed (***Table XI***).

Table XI: *Aerodynamic properties tested at 30 L/min inhalation flow rate in Andersen cascade impactor. MMAD = Mass median aerodynamic diameter and GSD = Geometric standard deviation. Data are presented as mean ± S.D., n=3. *Reference spray dried APIs.*

	MMAD (μm)		**GSD**	
	1st week	***10th week***	***1st week***	***10th week***
Non-porous formulations				
*MXP-SD**	*2.7±0.11*	*2.8±0.00*	*1.6±0.06*	*1.5±0.15*
MXP/LEU^{20}	2.9±0.03	2.9±0.08	1.3±0.06	1.3±0.22
MXP/LEU^{40}	2.6±0.07	2.7±0.06	1.5±0.03	1.5±0.02
MXP/LEU^{20}/PVA$^{2.5}$	2.7±0.31	2.9±0.22	1.4±0.13	1.4±0.26
MXP/LEU^{40}/PVA$^{2.0}$	2.7±0.07	2.9±0.13	1.4±0.12	1.5±0.07
Porous formulations				
*MX-SD**	*2.6±0.04*	*2.3±0.17*	*1.9±0.42*	*1.8±0.03*
MX/LEU/HA$^{0.30}$/AB$^{1.5}$	2.8±0.01	2.5±0.11	1.8±0.05	1.8±0.03
MX/LEU/HA$^{0.15}$/AB$^{1.5}$	2.6±0.12	2.5±0.03	1.8±0.08	1.7±0.05
MX/LEU/HA$^{0.30}$/AB$^{2.0}$	2.2±0.03	2.5±0.59	2.0±0.06	1.9±0.02
MX/LEU/HA$^{0.15}$/AB$^{2.0}$	2.5±0.23	2.4±0.59	1.7±0.06	1.6±0.01

Figure 17 demonstrates the relevance in pulmonary drug delivery of the large geometric diameter (>5 µm) and low density (<0.20 g/cm^3) porous formulations (***Fig. 17***). However, porous particles had larger geometric diameter (≤4.9 µm) than non-porous particles the MMAD values were similar. In case of non-porous formulations the increasing geometric diameter resulted in larger MMAD. However, in case of porous formulations, the MMAD was not increasing linearly with the geometric diameter. The difference between the geometric diameter and MMAD of non-porous formulations was just 1 µm, porous formulations had in average 2.8 µm differences in the same values. Different effects were observed comparing the excipient concentrations just in case of porous formulations. Porous particles with 2.0 mg/mL AB concentration exhibited lower MMAD (2.3–2.4 µm) than those with 1.5 mg/mL (2.6–2.7 µm) (***Fig. 19***). The higher amount of porogen affected positively the flowability of the particles thus resulted in lower aerodynamic diameter. By contrast, there was no significant difference between the MMAD of 0.15 and 0.30 mg/mL HA concentrations. In the used concentrations polymers (both HA and PVA) had no significant effect on the MMAD. In conclusion we were demonstrated that with appropriate density properties even larger sized particles can reach the same (or better) aerodynamic diameter and deposit in the lungs with higher amount.

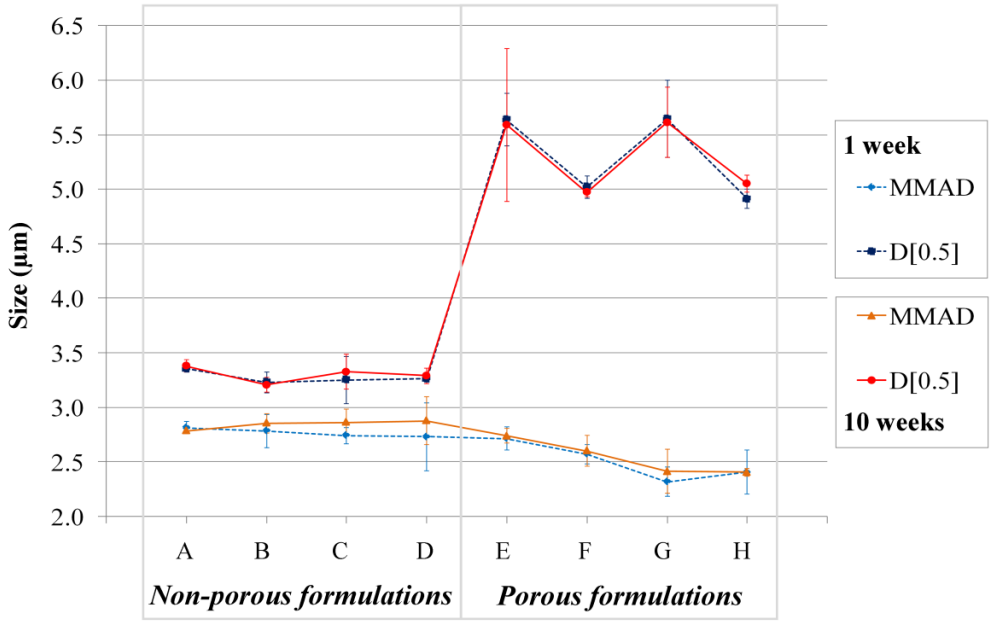


Figure 19: *Comparison of the diameters of the formulations (MMAD=mass median aerodynamic diameter and D[0.5]=median geometric diameter). A: MXP/LEU^{20}, B: MXP/LEU^{40}, C: MXP/LEU^{20}/PVA$^{2.5}$, D: MXP/LEU^{40}/PVA^{2}, E: MX/LEU/HA$^{0.3}$/AB$^{1.5}$, F: MX/LEU/HA$^{0.15}$/AB$^{1.5}$, G: MX/LEU/HA$^{0.3}$/AB^{2}, H: MX/LEU/HA$^{0.15}$/AB^{2}. Data are represented as mean ± S.D., n=3.*

5.7.2. In silico modelling

All samples were simulated *in silico* with the newest version of the Stochastic Lung Deposition Model using time-dependent 50.1 L/min average inhalation flow rates adopted from ***Chapman et al., 2011***. Figure 20 demonstrates the deposition fractions just of two samples of non-porous and two of porous formulations, as no significant differences were detected in the *in silico* properties of the similar formulation types. The simulations were performed for both $t_{b\text{-}h}$=5.0 and 10.0 s, to understand the effect on the aerosolization on aerodynamic parameters. There were significant differences between the longer and shorter breath-hold times (***Fig. 20***). Shortening the breath-hold from 10 to 5 s decreased the lung depositions with almost 5–12% (<47.5%). It was reported by Farkas et al. that particles reach the lungs deposit more efficiently by gravitational sedimentation if the breath-hold time is longer (***Farkas et al., 2017***). With $t_{b\text{-}h}$=10.0 s higher lung depositions were computed (>48.9% for both formulation types). However, breath-hold time length had no significant effect on the ET which was in a constant range of 22.2–25.2%. At the same time, the EXH were linearly decreasing by the increase of breath-hold time: if the higher amount of drug deposits in the lungs, then lower fraction remains for exhalation.

In the followings, the results of deposition simulations assuming $t_{b\text{-}h}$=10.0 s will be briefly discussed. The extrathoracic fraction presents the particle deposition in the upper airways (mouth, throat and trachea). Non-porous formulations deposit in the upper airways with 22.2–24.9% efficiency, which is almost the same than that of porous particles. However, porous formulations were larger than 5 μm just 21.1–25.7% of the inhaled particles were deposited in the extrathoracic region. It is worth mentioning that although there was no significant difference in the extrathoracic depositions of the two formulations, non-porous formulations had higher standard deviation values.

The *in silico* lung deposition values correlated well with the corresponding *in vitro* depositions at both formulation types. There was significant difference between the lung depositions of non-porous and porous particles. Non-porous particles were not reaching 50% deposition (maximum was 49.0% for MXP/LEU^{20}/PVA$^{2.5}$ after 10 weeks of storage), while porous particles had lung depositions constantly above 51% (maximum value was 52.2% for MX/LEU/HA$^{0.15}$/AB^{2}) (***Fig. 20***). Several commercially available DPIs were reported to be tested with the Stochastic Lung Model (even at higher inhalation flow rates), demonstrating a lower deep-lung deposition compared to the presented non-porous or porous DPI formulations (***Jókai et al., 2015***; ***Jókai et al., 2016***).

The exhaled fraction is a relevant output parameter of the *in silico* modelling as the official *in vitro* aerodynamic testing methods are not validated to provide this data. In this work, the exhaled fraction was calculated as the difference between the inhaled amount and the sum of the amounts deposited in the extrathoracic airways and the lungs. The porous particles had lower EXH (23.6–27.3%) than the non-porous particles (26.0–27.5%). No significant differences were detected in terms of extrathoracic and lung depositions and the exhaled fractions between the formulations after 1 and 10 weeks of storage.

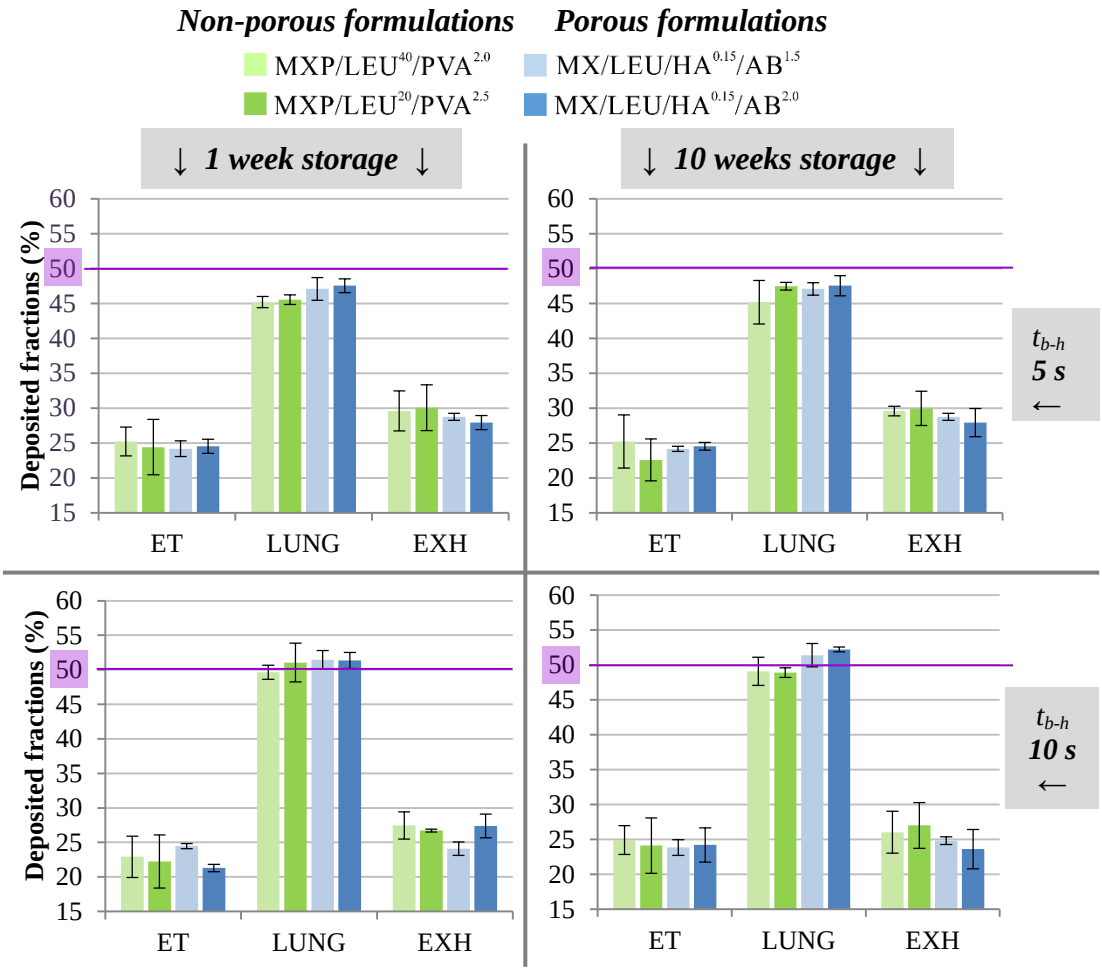


***Figure 20**: In silico modelling results (1 and 10 weeks after storage) using spirometry data of 7 COPD patients: inhaled volume (IV) 1.70 L, inhalation time (t_{in}) 2.04 s, breath-hold time ($t_{b\text{-}h}$) 5 and 10 s, exhalation time (t_{ex}) 3.0 s, 50.1 L/min average airflow rate (time-dependent inhalation). ET=extrathoracic deposition, LUNG= lung deposition and EXH=exhaled fraction. Data are presented as mean ± S.D., n=3.*

6. CONCLUSION

In accordance with our research goals, **carrier-free DPIs** were produced for the local treatment of pulmonary inflammatory diseases. The optimal formulations of carrier-free DPIs have high practical relevance in pulmonary drug delivery:

- carrier-free formulations aimed to reduce the intrinsic cohesion of the particles, increase dispersion and delivery from the inhaler, thus emitting the use of large carriers for drug delivery and reducing the side effects of particles deposited in the upper airways.

It was clarified that MXP has a similar effect on **A549 cells** viability as MX and both can be safely used for inhalation up to 0.1 mg/mL concentration.

The aimed **"spray drying from solution" technology** was accomplished with two feasibility strategies. The technologies using the novel MXP salt form and applying MX solubility increase (with pH shift) eliminate the use of organic solvents for dissolving the active agents:

- the use of high water solubility of **MXP** simplifies sample preparation: with 70±5 °C heated purified water, the drug concentration could reach 20 mg/mL MXP concentration in each formulation, while
- the **MX** aqueous solution was achieved with pH adjustment (at pH 8.0±0.1) to facilitate the particle formulation process and reach 1.5 mg/mL MX concentration (in sodium salt form) in each formulation.

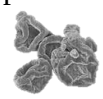
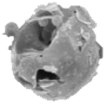
The formulation and analyses protocol was based on a comparison study of the non-porous and porous formulations regarding their spray drying yield (>60% for both technologies), physicochemical properties and aerodynamic behaviour (***Table XII***):

- the planned **non-porous formulations** containing MXP reached the aimed narrow size distribution (Span<1.9) in the inhalable 3.0–3.8 μm geometric diameter range and spherical-like morphology. The density of these particles was 0.32–0.42 g/cm^3, while
- the planned **porous formulations** (LPP technique) containing MX reached the aimed narrow size distribution (Span <2.0), particles had spherical morphology, reached geometric diameter larger than 5 μm (up to 5.7 μm) and density lower than 0.17 g/cm^3.

The **aerodynamic behaviour** of the formulations showed no significant difference in the course of *in vitro-in silico* evaluation after 1 and 10 weeks of storage (***Table XII***):

- the ***in vitro*** aerodynamic properties performed at 30 L/min low inhalation flow rate exceed the values of commercially available formulations. Thanks to the better aerosolization of porous particles, these formulations showed the highest drug emission and deposition values (EF >76 % and FPF >54 %). Despite the large geometric size of porous formulations, the aerodynamic diameter were similar to those of the non-porous formulations, falling in the inhalable range (with MMAD 2.2–3.0 μm), while
- the ***in silico*** aerosolization properties of the formulations were established at COPD patients' time-dependent inhalation flow rate (average 50.1 L/min). Besides the low extrathoracic (<25%) and high lung deposited fraction (>46%), the exhaled fraction could also be determined (<27.4%, $t_{b\text{-}h}$=10 s). The *in silico* lung deposition values correlated well with *in vitro* measured FPF: showing higher lung deposited fractions in both cases of formulation techniques.

Table XII: *Summary of the presented study. The most important comparison properties of the prepared carrier-free DPI formulations.*

		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, dense particles 	spherical, porous/hollow structure 
Geometric size		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 aerodynamic 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	1.33–1.52	1.66–2.01
In silico depositions $t_{b\text{-}h}$=10 s	ET	22–24%	21–25%
	LUNG	48–51%	51–52%
	EXH	26–27%	23–27%

The inhibition of inflammation with the appropriate inhalable NSAIDs could be one of the possible cures affecting more than 500 million people worldwide. The presented well controlled DPI particles could offer new possibilities in the use of meloxicam in inhalation therapy both alone and in combination products. The aimed carrier-free DPIs containing MXP and MX may offer safe treatment of **lung inflammations** (e.g. pulmonary fibrosis, COPD). Thanks to the high aerodynamic properties, the presented carrier-free DPI formulations containing MXP and MX may offer effective local treatment for lung inflammation diseases (***Durham et al., 2015***).

Both the non-porous and the large porous formulations prepared were found to have good aerodynamic properties. The optimized formulation technology was essential for the high aerodynamic performance which affects the bioavailability of the pharmaceutics.

The combination of *in vitro* assessment and *in silico* modelling demonstrated better aerodynamic behaviour than that of the presently commercially available DPI products. The use of *in vitro-in silico* combination analyses gives 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.

New findings/practical relevance of the work:

- A novel "spray drying from aqueous solution" technology was developed to design carrier-free meloxicam (MXP or 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 carrier-free DPIs as non-porous particles with the salt form of MX (MXP), and porous formulation with the pH adjustment of MX.

- Both the non-porous and the large porous meloxicam 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 meloxicam containing DPI particles could offer new possibilities in the use of NSAIDs in inhalation therapy both alone and in combination products for the local treatment of lung inflammation diseases, such as pulmonary fibrosis and COPD.

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3. sz. melléklet: nyilatkozat az értekezés eredetiségéről

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Név: ***Dr. Chvatal Anita***

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