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Summary of the Ph.D. thesis

DEVELOPMENT OF NOVEL FORMULATED CIPROFLOXACIN HYDROCHLORIDE CONTAINING DRY POWDER INHALATION SYSTEM

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1. INTRODUCTION

Inhalation therapy dates back a long time, but research and product development for pulmonary drug delivery (PDD) has been focused on the last two to three decades at least. Accordingly, several companies and research groups have specialized in this field. The products and research available on the market are still typically focused on local therapy (e.g. asthma, cystic fibrosis (CF), tuberculosis, etc.). In particular, the use of antibiotics in inhalation therapy has a lot of potentials, as bacterial infections typical of many lung diseases can be treated locally with a remarkable reduction in systemic side effects compared to *oral* administration. There are not yet many inhaled products containing antibiotics on the market, but several promising formulations have been published. There is great potential for the inhaled use of ciprofloxacin hydrochloride (CIP) as a broad-spectrum fluoroquinolone antibiotic.

There are four main categories of PDD implementation: nebulizers, soft mist inhalers (SMIs), pressurized metered-dose inhalers (pMDIs), and dry powder inhalers (DPIs). Among these, the development of DPI systems has recently received the greatest focus due to its many advantageous features. On the one hand, in the case of DPIs, traditional carrier-based systems are distinguished, where micronized drug particles create an interactive physical mixture with large carrier particles. The micronized drug particles are then swept off from the surface of the large carrier during inhalation. The other group consists of carrier-free DPIs, where different manufacturing methods and applied excipients allow good aerodynamic properties. Both groups of DPIs mentioned above have advantageous properties and development potential, but the combination of their beneficial properties indicates a new development line that is still unexploited.

The efficiency of DPI products is influenced - in addition to the DPI formulation - by the applied DPI device, since the design and characteristics of the delivery system, the adhesion between the DPI formulation and the device also contributes to the achievement of the appropriate aerodynamic result. Nevertheless, the properties of DPI capsules can also influence the aerosolization, aerodynamics and stability of inhalation powders, and are therefore not only used for packaging of formulations. Patients also play a major role in the aerosolization and lung deposition outcome of DPI powders, for example in terms of breathing maneuver and breath-holding time. Overall, the development of DPI dosage forms is challenging, but there are many untapped opportunities.

Abbreviations: *AFM – Atomic Force Microscope; API – Active Pharmaceutical Ingredient; APSD – aerodynamic particle size distribution; BH – breath-hold time; CF – cystic fibrosis; CIP – ciprofloxacin hydrochloride; D[0.5] – Geometric diameter; DPI – Dry powder inhaler; EF – emitted fraction; ET – extrathoracic airways; EtOH – ethanol; EXH – exhalation fraction;* F_{adh} *– adhesion force; FPF – fine particle fraction; ICH – The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; IH 70 – Inhalac® 70; GEL – Gelatine; GEL-PEG – Gelatine-polyethylene glycol; HPMC – Hydroxypropyl methylcellulose; LUNG – bronchial and acinar parts; MgSt – magnesium stearate; MMAD – mass median aerodynamic diameter; MXP – Meloxicam potassium; NaSt – sodium stearate; PDD – pulmonary drug delivery; pMDI – pressurized metered-dose inhaler; RH – relative humidity;* R_{RMS} *– Root means square roughness; RSC – Residual solvent content; SEM – Scanning Electron Microscope; SMI – Soft Mist Inhaler; spd – spray dried/spray drying; XRPD – X-ray Powder Diffraction;* W_{adh} *– adhesion work;* W_c *– cohesion work*

2. AIMS OF THE WORK

The goal of the Ph.D. work was to develop and investigate a novel formulated DPI microcomposite containing CIP as an antibiotic agent, which combines the advantages of traditional, carrier-based, and carrier-free formulations and provides adequate stability. The ultimate aim was to create a well-adapted DPI drug formulation for the treatment of cystic fibrosis and chronic bronchitis. The main steps of the work were as follows:

I. Literature review of the benefits of PDD compared to the *oral* administration route. Exploration of the similar pathophysiological course of CF and chronic bronchitis and the potential for antibiotic therapy intervention in this progressive process. Identification of PDD options and collection of inhaled antibiotic formulations on the market. Furthermore, to collect factors influencing the effectiveness of the delivery of DPI systems and to explore their potential.

II. Development of a **carrier-based** CIP-containing DPI system where the carrier particles are surface-treated with magnesium stearate (MgSt) and the drug particles were blended on the surface of these particles are prepared by prior spray drying (spd). In this way, a sample with better *in vitro* lung deposition results than the traditional carrier-based DPI formulations on the market can be expected.

III. To study how different solvent proportions used in the stock solution during the preparation of CIP-containing **carrier-free** DPI samples, influences the habit of these powders and consequently their *in vitro* aerodynamic results. The purpose is to apply the results to select the optimal solvent composition for this drug. This segment seeks to shed a new perspective on published experience, using new numerical concepts, and contexts to provide new explanations and expand knowledge on the subject. In addition, preparation, physicochemical, and *in vitro* lung deposition studies of co-spd DPI formulations containing the active ingredient CIP and sodium stearate (NaSt) as an excipient in different concentrations using the optimal solvent mixture described above. Thereby further development of the carrier-free DPI direction.

IV. Development, physical / *in vitro* lung deposition / *in silico* lung simulation investigations, and short-term stability testing of **novel formulated** DPI microcomposite containing CIP, through the integration of the experience gained from the improvement of traditional carrier-based and carrier-free DPI formulations described above. Furthermore, the development of the final dosage form of this above-mentioned DPI sample, which involves testing in different DPI capsule types and a more extended 6-month stability study according to the ICH (The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) guideline.

3. MATERIALS ANF METHODS

3.1. Materials

3.1.1. Active pharmaceutical ingredient (API)

Ciprofloxacin hydrochloride (CIP) – second-generation fluoroquinolone antibiotic – was applied and received by TEVA Pharmaceutical Industries Ltd. (Debrecen, Hungary).

3.1.2. Excipients

α-lactose monohydrate, Inhalac® 70 (IH70) (Meggle Group GmbH., Wasserburg am Inn, Germany) was applied as a carrier. Magnesium stearate (MgSt) (Sigma Aldrich, Budapest, Hungary) was used to treat the surface of IH70. Sodium stearate (NaSt) (Alfa Aesar, Heysham, United Kingdom) was used as an excipient in the co-spd process. The 96% ethanol (EtOH, AppliChem GmbH, Darmstadt, Germany) – solvent – was used to modify the physical properties of the produced microcomposites.

3.2. Methods

3.2.1. Preparation of the samples

The following describes how traditional carrier-based, carrier-free and novel formulated DPI samples were produced. Since the carrier-free samples are in many cases also components of carrier-based formulations, their preparation is presented earlier.

3.2.1.1 Formulation of carrier-free composites

The DPI samples were made up from aqueous solutions of CIP (Table 1.). The CIP concentration in the stock solutions was 0.015 g/mL in all cases. In one of the samples, the stock solution did not include EtOH, in the other cases EtOH was used as a liquid excipient at various concentrations (5%, 10%, 20% and 30%) near distilled water and the used temperature was 65 °C. Samples containing NaSt were also applied with the same amount of API as described above, which was first solubilized in distilled water. In parallel, the corresponding amount of NaSt was solved in EtOH. Then, the two above-mentioned solutions were mixed in a ratio of 7:3 (for NaSt-containing samples, in the stock

solution 30% EtOH concentration was used in all cases). The (co-)spd process was carried out using a Büchi B-191 (Mini Spray Dryer, Büchi, Switzerland) and the parameters were as follows: inlet heating temperature 130 °C, outlet temperature about 80 °C, drying air flow rate 75%, sample pump speed 5% and compressed air flow rate 600 L/h.

Table 1. *Samples for carrier-free formulation development with CIP content.*

Samples	CIP (g)	NaSt (g)	EtOH (g)	H_2O (g)
$CIP_{spd}EtOH^{0}$	3.0	-	-	ad 200
$CIP_{spd}EtOH^{5}$	3.0	-	10.0	ad 200
$CIP_{spd}EtOH^{10}$	3.0	-	20.0	ad 200
$CIP_{spd}EtOH^{20}$	3.0	-	40.0	ad 200
$CIP_{spd}EtOH^{30}$	3.0	-	60.0	ad 200
$CIP_{spd}EtOH^{30}NaSt^{0.5}$	3.0	0.0151	60.0	ad 200
$CIP_{spd}EtOH^{30}NaSt^{1.0}$	3.0	0.0303	60.0	ad 200
$CIP_{spd}EtOH^{30}NaSt^{2.0}$	3.0	0.0606	60.0	ad 200

3.2.1.2. Preparation of the carrier-based formulations

The raw CIP (D [0.5]: 10.51 μm) particle size was reduced by sieving (mesh size: 25μm) to allow the production of traditional carrier-based formulations as a reference, by reducing the applied API particles to a size range relevant for inhalation therapy. The carrier-based DPI formulations were prepared using CIP_{sieved} or CIP_{spd} particles and carrier/surface-modified carrier, blended in a 1:10 mass ratio by Turbula mixing (T2F Turbula System Schatz; Willy A. Bachofen AG Maschinenfabrik, Basel, Switzerland) with a 30 min mixing time at 60 rpm. The surface modification of the carrier (IH70) was completed by using 2.0 w/w% of MgSt relative to the final formulations – Table 2. – with Turbula blending for 4 h. Thus, MgSt can form a thin film coating the surface of the IH, which can modify its surface properties.

Table 2. *Samples for carrier-based DPI development with CIP content*

Samples	**CIP_{sieved}** (w/w %)	**$CIP_{spd}EtOH^{10}$** (w/w %)	**$CIP_{spd}EtOH^{30}$ $NaSt^{0.5}$**(w/w %)	**IH 70** (w/w %)	**MgSt** (w/w %)
CIP_{sieved}+IH70	9.09	-	-	90.91	-
CIP_{sieved}+IH70_MgSt	9.09	-	-	88.91	2.00
$CIP_{spd}EtOH^{10}$+IH70	-	9.09	-	90.91	-
$CIP_{spd}EtOH^{10}$+IH70_MgSt	-	9.09	-	88.91	2.00
$CIP_{spd}EtOH^{30}NaSt^{0.5}$+IH70_MgSt (*novel formulated DPI)*	-	-	9.09	88.91	2.00

3.2.2. Determination of blend uniformity and real drug content

The real API content was also investigated for every DPI microcomposites. These were dissolved in distilled water and the CIP content was calculated using a UV/VIS spectrophotometer (ATIUNICAM UV/VIS spectrophotometer, Cambridge, United Kingdom) at a wavelength of 276 nm. The linearity of the CIP in this medium at the above-mentioned wavelengths was determined in advance. The linearity of the calibration curve was y = 0.0736x. The unit of the slope was mL/μg.

3.2.3. Stability tests

Stability experiments were carried out in a Binder KBF 240 device (Binder GmbH, Tuttlingen, Germany) with a constant climate chamber. An electronically operated APT.line^{TM} line preheating chamber and cooling system provided temperature precision and repeatability of results over a temperature range of 10 to 70 °C and a relative humidity (RH) range of 10 to 80 %. Two types of stability tests were performed (Table 3.).

Table 3. *Details of the stability tests.*

Conditions for the stability test	Duration of the stability test	Investigated formulations	Applied DPI capsule types
T: 25 ± 2 °C RH: 50 ± 5%	1 month (short-term)	CIP_{sieved}+IH70 $CIP_{spd}EtOH^{30}NaSt^{0.5}$ $CIP_{spd}EtOH^{30}NaSt^{0.5}$ +IH70_MgSt	GEL
T: 40 ± 2 °C RH: 75 ± 5 %	6 months (long-term)	$CIP_{spd}EtOH^{30}NaSt^{0.5}$ $CIP_{spd}EtOH^{30}NaSt^{0.5}$ +IH70_MgSt	GEL, GEL-PEG, HPMC

3.2.4. Characterization using Light Microscope

The holes created by punching the capsules were recorded in shape and area using a Leica image analyzer (Leica Q500MC, LEICA Cambridge Ltd., Cambridge, United Kingdom) at 4x magnification. For each capsule type, ten repeats were carried out in all cases.

3.2.5. Thermogravimetry measurements

The Mettler Toledo STARe (Mettler Inc., Schwerzenbach, Switzerland) was applied to determine the RSC of DPI capsule types and DPI formulations. Regarding thermogravimetric tests, 3-5 mg of sample per capsule was weighed into 40 μl aluminum crucibles, and the temperature dependence of the mass change of the samples was observed between 25-350 °C at a heating rate of 10 °C / min under nitrogen gas flow. The weight loss up to 110 °C was due to the water leaving the sample.

3.2.6. X-ray powder diffraction (XRPD)

The structural identification of the samples was detected with a BRUKER D8 Advance X-ray powder diffractometer (Bruker AXS GmbH, Karlsruhe, Germany). Cu K λI radiation (λ = 1.5406 Å) was the radiation source. The investigated solid-state samples were monitored at 40 kV and 40 mA, with an angular range of 3°-40° 2-Theta, a step time of 0.1 s/step, and a step size of 0.01°. The DIFFRACT plus EVA software (Bruker, Brussels, Belgium) was utilized to assess the results.

3.2.7. Particle size distribution

For the determination of the particle size distribution of the samples, laser diffraction was applied (Malvern Mastersizer Scirocco 2000, Malvern Instruments Ltd., Worcestershire, United Kingdom). The dispersion air pressure was 2.0 bar. Three measurements were carried out in repetition. The particle size distribution was characterized by the values D [0.1], D [0.5], and D [0.9].

3.2.8. Morphology by Scanning Electron Microscope (SEM)

The shape, surface features and estimated size of the raw materials/formulations were studied by SEM (Scanning Electron Microscope – Hitachi S4700, Hitachi Scientific Ltd., Tokyo, Japan). The range of air pressure used was 1.3-13.0 MPa. Samples were coated with gold-palladium (90 s) in an argon atmosphere with the use of a gold sputtering module within a high vacuum evaporator.

3.2.9. Topology by Atomic Force Microscope (AFM), and the expressed values

The imaging was performed with a FLexAFM atomic force microscope supported by C3000 control software (Nanosurf AG, Liestal, Switzerland). To extend the dynamic force measurement mode, uncoated TAP-300-Al-G cantilevers (BudgetSensors, Sofia, Bulgaria) with a nominal resonance frequency of 300 kHz were applied in phase-contrast mode. Data were processed and interpreted via Gwyddion 2.55 software (Czech Metrology Institute, Brno, Czech Republic). Root means square roughness (R_{RMS}) was determined according to Equation 1. by running the Gwyddion software. In the case of each sample, at least five particles and an area of 0.5 μm x 0.5 μm per individual particle were analyzed at least at three separate locations.

$$R_{RMS} = \sqrt{\frac{1}{n}\sum_{i=1}^{n} y_i^2} \quad (1)$$

In this equation, 'n' is the number of data points in the topographic profile and 'y_i' is the distance of the asperities (i) from the midline. The roughness (%) value for the particle size was obtained from the average R_{RMS} ($R_{RMS\,(average)}$) and the average diameter (d) – the average of 10 diameters in various orientations – for a given particle, using the following equation:

$$\text{Roughness } \% = \frac{R_{RMS\,(average)}}{d} \times 100 \quad (2)$$

Moreover, the above-mentioned software makes it possible to measure only the single depressions/dimples of the particle surface. In the case of each particle, a minimum of three dimples was used as the basis for determining the average values (depth and width of the dimples (average)). By obtaining these values, and also knowing the value of d for the particle as discussed above, the depth and width of the dimples relative to the particle size were calculated (Equations 3 and 4). For each sample, analyses were carried out for at least five particles.

$$\text{Depth of the dimples } \% = \frac{Depth\ of\ the\ dimples_{(average)}}{d} \times 100 \quad (3)$$

$$\text{Width of the dimples } \% = \frac{Width\ of\ the\ dimples_{(average)}}{d} \times 100 \quad (4)$$

3.2.10. Studying the interparticle interactions

To investigate the interparticle interactions between the components of the samples, pastilles were created using 100 mg of each substance and 1 tonne of compressive force (Perkin Elmer hydraulic press, Waltham, United States). The contact angle (Θ) was established using a Dataphysics OCA 20 instrument (Dataphysics Inc. GmbH, Germany). Three pastilles per sample were dropped with a polar liquid medium (distilled water) and the other three pastilles were dropped with a dispersion liquid medium (diiodomethane). Simultaneously with the dripping, the instrument was set to record at a time interval of 1-25 s. In this way, the contact angle of the two different applied liquids was obtained, determined always in relation to the same second. Surface free energy (γ_s) of the components, which is made up of two parts: a disperse (γ_s^d) and a polar part (γ_s^p), that is ($\gamma_s = \gamma_s^d + \gamma_s^p$), was calculated using the Wu formula. The cohesion work (W_c) is defined as twice the surface free energy The adhesion work (W_{adh}) can be established between two different substances (denoted by the numbers 1 and 2) and is obtained from the value of the disperse (γ_s^d) and the polar component (γ_s^p) of the substance, in this formula γ^d and γ^p. The W_{adh} can be calculated in the following way:

$$W_{adh} = 4\left[\frac{\gamma_1^d\gamma_2^d}{\gamma_1^d + \gamma_2^d} + \frac{\gamma_1^p\gamma_2^p}{\gamma_1^p + \gamma_2^p}\right] \quad (5)$$

The Derjaguin approach was used to determine the adhesion force (F_{adh}):

$$F_{adh} = 2\pi\left(\frac{R_A R_B}{R_A + R_B}\right)W_{adh} \quad (6)$$

where R_A and R_B are the radii of the particles A and B, respectively, between which the adhesion interaction is studied.

The spreading coefficient (S_{12}) – a dimensionless value – shows the probability of one material (1) on the surface of the second component (2). It is used to characterize the distribution in binary systems. The spread is favorable if it has a positive value and a high number. In this case, the API particle spreading over the surface of either a carrier or a surface-modified carrier is investigated.

3.2.11. In vitro aerodynamic test

The Andersen Cascade Impactor (Copley Scientific Ltd., Nottingham, United Kingdom) was used as an *in vitro* testing tool to characterize the aerodynamic particle size distribution (APSD) of the formulations. Discs of the impactor were coated with a mixture of Span® 80 and cyclohexane (1:99) and left to dry. The applied flow rate (28.3 ± 1 L/min or 60 ± 1 L/min) for the measurements was generated using a vacuum pump (High-capacity Pump Model HCP5, Critical Flow Controller Model TPK, Copley Scientific Ltd., Nottingham, United Kingdom), and monitored with a mass flow meter (Flow Meter Model DFM 2000, Copley Scientific Ltd., Nottingham, United Kingdom). In the *in vitro* assay, three capsules from a studied sample were applied in a single measurement, using the Breezhaler® (Novartis, Basel, Switzerland). For each single used capsule, the inhalation time was set as related to the flow rate (28.3 L/min or 60 L/min) so that the inhaled volume was always 4 L. After each *in vitro* examination, the inhaler, the applied DPI capsules, and the components of the impactor were rinsed with distilled water. The quantity of API deposited was detected using an UV/VIS spectrophotometer (ATI-UNICAM UV/VIS spectrophotometer, Cambridge, United Kingdom) with a 276 nm wavelength. From the above data, the terms describing the *in vitro* aerodynamic behavior of the samples can be calculated: emitted fraction (EF), fine particle fraction (FPF), and mass median aerodynamic diameter (MMAD).

3.2.12. In silico assessment

Quantification of inhaled API deposition in the airways was carried out with the help of the stochastic lung model. In this study, the most recent version of the model was used and it was validated for medical aerosols. Patients' respiratory data and the APSD values of the studied formulations were the key inputs to the deposition model.

3.2.13. Statistical Analyses

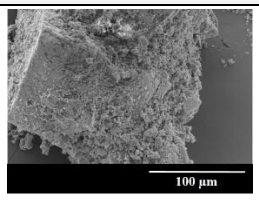
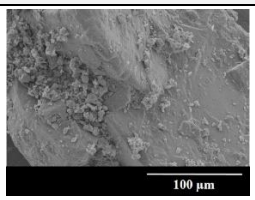
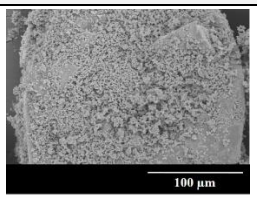
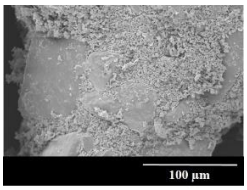
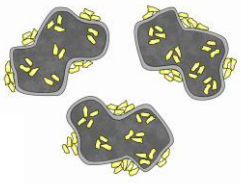
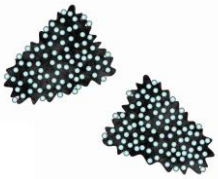
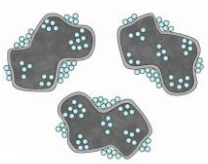
Statistical analyzing was performed using t-test computations with a significance level of 0.05 and one-tailed hypothesis using Social Science Statistics available online. All reported data represent the ± standard deviation of three replicate tests (n = 3).

4. RESULTS

4.1. Development of CIP containing traditional carrier-based DPI samples

An innovative formulation sample with improved *in vitro* aerodynamic (FPF) performance has been prepared, to our knowledge not yet reported, which already contains a mechanical dry coated carrier with MgSt and spd API on its surface ($CIP_{spd}EtOH^{10}$+IH70_MgSt). This is the initial improvement of the development line described in this thesis, which can be considered as the starting point for the combination of the two systems in our case, which still belongs to the development of traditional carrier-based DPI systems. Moreover, through the study of interparticle interactions (Table 4.), a correlation was found between the physical properties and *in vitro* lung deposition results of interactive physical mixtures.

Table 4. *The SEM recording and interparticle interactions of carrier-based DPI samples.*

Carrier-based samples	**CIP_{sieved} +IH70**	**CIP_{sieved} +IH70_MgSt**	**$CIP_{spd}EtOH^{10}$ +IH70**	**$CIP_{spd}EtOH^{10}$ +IH70_MgSt**
SEM				
Schematic pictures				
W_{adh} (mN/m)	108.26	78.27	98.72	73.23
F_{adh} (mN)	$1.690*10^{-3}$	$1.216*10^{-3}$	$0.596*10^{-3}$	$0.440*10^{-3}$
S_{21}	1.64	-45.59	16.67	-18.36

In the case of meloxicam potassium (MXP), this development was similarly carried out (only the API was changed in the manufacturing process, and the production and testing methods were the same), and the in vitro aerodynamic results of the samples showed the same trend.

4.2. Development of CIP containing carrier-free DPI formulations

4.2.1. EtOH concentration optimisation in the stock solution

This work introduces new expressions for particle size-related roughness, depth and width of dimples, and correlation of MMAD/D [0.5] ratio with W_c (Figure 1.), which to the best of our knowledge have not been published before, resulting in gap-filling findings.

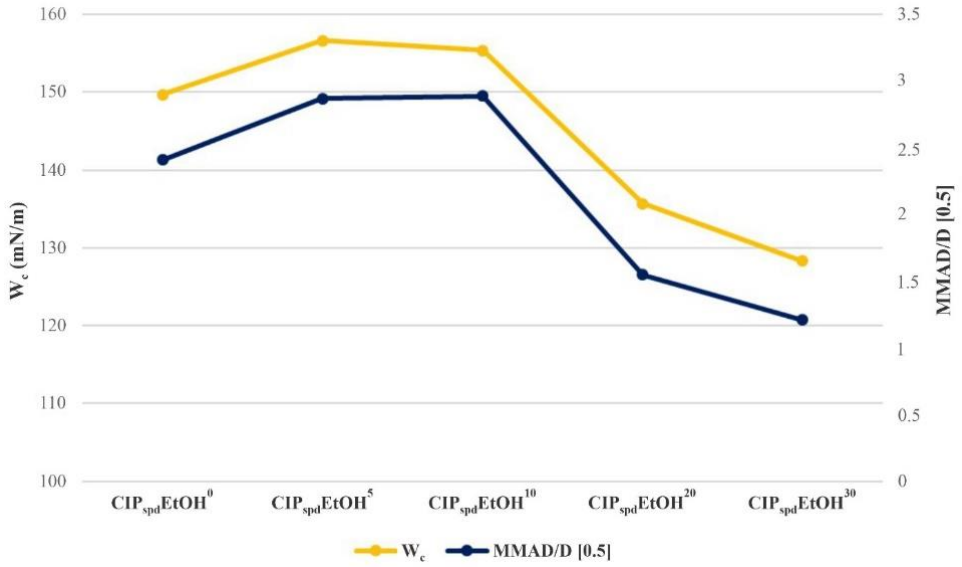


***Figure 1**. Relationship between the trends of W_c and MMAD/D [0.5] values.*

As a result, different ratios of solvent mixtures can be put into a new perspective as different EtOH concentrations affect the habit of the DPI formulations and therefore their *in vitro* aerodynamic performance. Based on these findings, it became clear why the stock solution of the tested DPI formulation containing 30% EtOH ($CIP_{spd}EtOH^{30}$) achieved the best FPF result (Table 5.).

***Table 5.** In vitro aerodynamic properties of the formulations measured at 28.3 L/min flow rate.*

Samples	FPF (%)	MMAD (µm)	EF (%)
$CIP_{spd}EtOH^{0}$	23.58 ± 0.73	7.62 ± 0.13	86.26 ± 0.44
$CIP_{spd}EtOH^{5}$	11.96 ± 0.16	12.03 ± 0.08	89.57 ± 0.31
$CIP_{spd}EtOH^{10}$	13.05 ± 0.25	10.18 ± 0.16	78.13 ± 0.83
$CIP_{spd}EtOH^{20}$	28.51 ± 0.43	5.83 ± 0.03	75.80 ± 0.65
$CIP_{spd}EtOH^{30}$	34.39 ± 0.54	5.21 ± 0.11	87.12 ± 0.39

4.2.2. Optimization of NaSt concentration

The use of NaSt in co-spd manufacturing of DPIs affects the size and shape of the particles, thereby having a positive effect on the *in vitro* aerodynamic results. This has been confirmed for two drug materials (CIP and MXP) in our cases, however, it depends on the API in which NaSt concentration is applied to achieve the best *in vitro* aerodynamic results. In the case of CIP, the use of 0.5% NaSt ($CIP_{spd}EtOH^{30}NaSt^{0.5}$ sample) is optimal based on the investigations of our studies.

4.3. Development of a pulmonary dosage form of the novel formulated DPI microcomposite

4.3.1. Comparison of a novel formulated DPI formulation with traditional carrier-based and developed carrier-free DPI samples

By combining the advantages of traditional carrier-based and carrier-free DPI improvements, the novel formulated DPI ($CIP_{spd}EtOH^{30}NaSt^{0.5}$+IH70_MgSt) was a successful development, since the cohesive-adhesive balance can be attained by particle engineering of the API and applied of excipients, and therefore has the best *in vitro* aerodynamic performance of all our developments, further confirmed by *in silico* simulation (Figure 2.). In addition, this formulation embodies a new/third category of DPIs besides the traditional carrier-based and carrier-free formulations, which is a novelty in the literature. Moreover, *in silico* simulation has shown that the patient can further improve lung deposition results with appropriate inhalation technique.

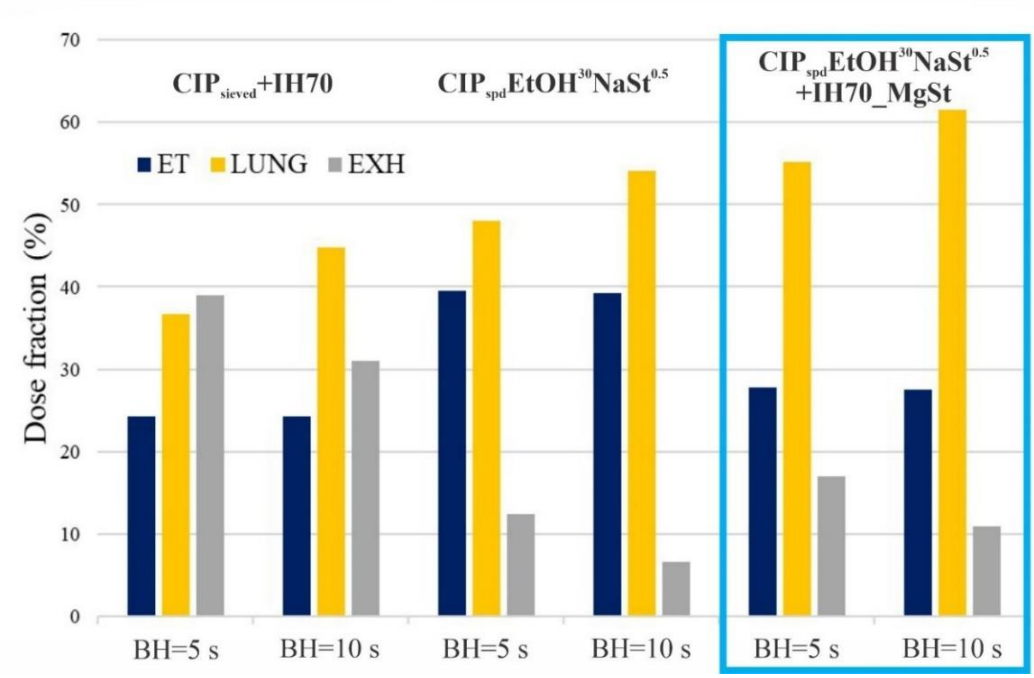
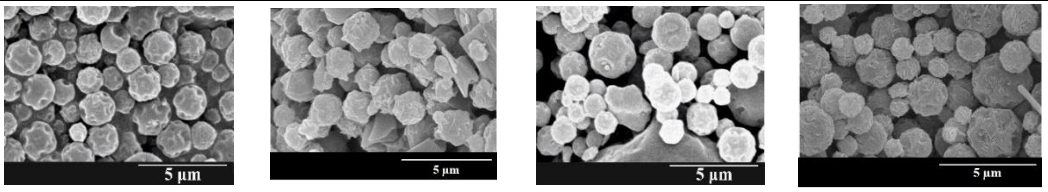


Figure 2. *Comparison of three formulation methods by in silico simulation using different breath-hold times (BHs) (ET: extrathoracic airways, LUNG: bronchial and acinar parts, EXH: exhalation fraction).*

4.3.2. Short-term stability results of the novel formulated DPI sample

For the three samples described in the previous section, i.e. a traditional carrier-based (CIP_{sieved}+IH70), the best *in vitro* lung deposition performance carrier-free ($CIP_{spd}EtOH^{30}NaSt^{0.5}$) and a novel formulated DPI ($CIP_{spd}EtOH^{30}NaSt^{0.5}$+IH70_MgSt), short term stability testing was performed at room temperature under the conditions detailed in section 3.2.3. After storage, the novel formulated DPI had the best MMAD and FPF results after 1 month (Table 6.). In terms of interparticle interactions, the W_{adh} of CIP_{sieved}+IH70 remained high during the stability study, while the W_c of $CIP_{spd}EtOH^{30}NaSt^{0.5}$ increased considerably, suggesting that the latter formulation aggregates more easily (as confirmed by SEM images, recrystallization is visible). The results show that $CIP_{spd}EtOH^{30}NaSt^{0.5}$+IH70_MgSt has better stability than the carrier-free formulation (although the used API particles were produced in the same way), so the use of a carrier surface-treated with MgSt improved not only the lung deposition results but also the stability compared to $CIP_{spd}EtOH^{30}NaSt^{0.5}$.

Table 6. *SEM records and in vitro aerodynamic results at 28.3 L/min flow rate of the carrier-free ($CIP_{spd}EtOH^{30}NaSt^{0.5}$) and novel formulated ($CIP_{spd}EtOH^{30}NaSt^{0.5}$+ IH70_MgSt) DPI formulations before storage and after 1-month short-term stability test.*

	$CIP_{spd}EtOH^{30}NaSt^{0.5}$		**$CIP_{spd}EtOH^{30}NaSt^{0.5}$+IH70_MgSt**	
	Before storage	1 month	Before storage	1 month
SEM				
FPF (%)	54.27 ± 2.75	30.22 ± 1.82	63.75 ± 1.21	47.12 ± 0.78
MMAD (µm)	4.14 ± 0.18	6.54 ± 0.05	3.47 ± 0.02	5.47 ± 0.35
EF (%)	76.99 ± 3.32	92.32 ± 0.19	90.45 ± 1.80	89.46 ± 1.12

In silico lung modeling was performed for the novel formulated DPI, which correlated with the *in vitro* aerodynamic results. It should be highlighted that the ET dose fraction value of this sample was still below 30% after one month, while the other two samples showed worse results when freshly prepared. Finally, it can be concluded that the novel formulated DPI achieved better *in vitro-in silico* aerodynamic results than the traditional carrier-based CIP_{sieved}+IH70 and carrier-free $CIP_{spd}EtOH^{30}NaSt^{0.5}$ formulations even after 1 month of storage due to its favorable properties.

4.3.3. Long-term stability results of the novel formulated DPI sample in different capsule types

As a follow-up to the stability study described in section 4.3.2, and as the final step in the development of the dosage form, $CIP_{spd}EtOH^{30}NaSt^{0.5}$ and $CIP_{spd}EtOH^{30}NaSt^{0.5}$+IH70_MgSt (for further formulation designations: 1 and 2), a 6-month stability study was performed according to the ICH guideline at 40 ± 2 °C, 75 ± 5 % RH, and for all formulations also in GEL, GEL-PEG, HPMC DPI capsules to analyze the effect of DPI capsule types on their stability. The stability of the applied DPI capsules was also separately investigated under the above-mentioned conditions.

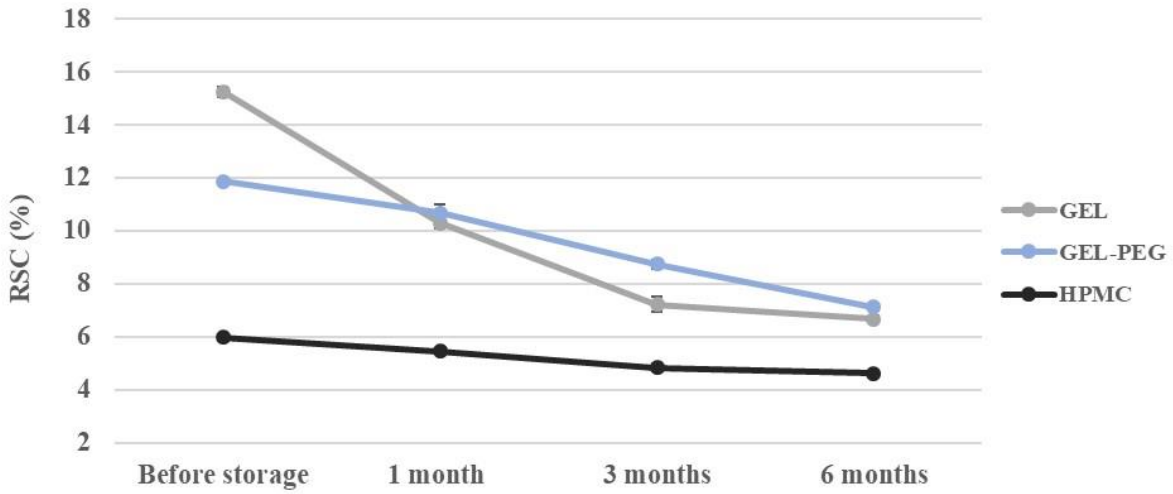


Figure 3. *RSC values of the capsule walls during the long term stability test.*

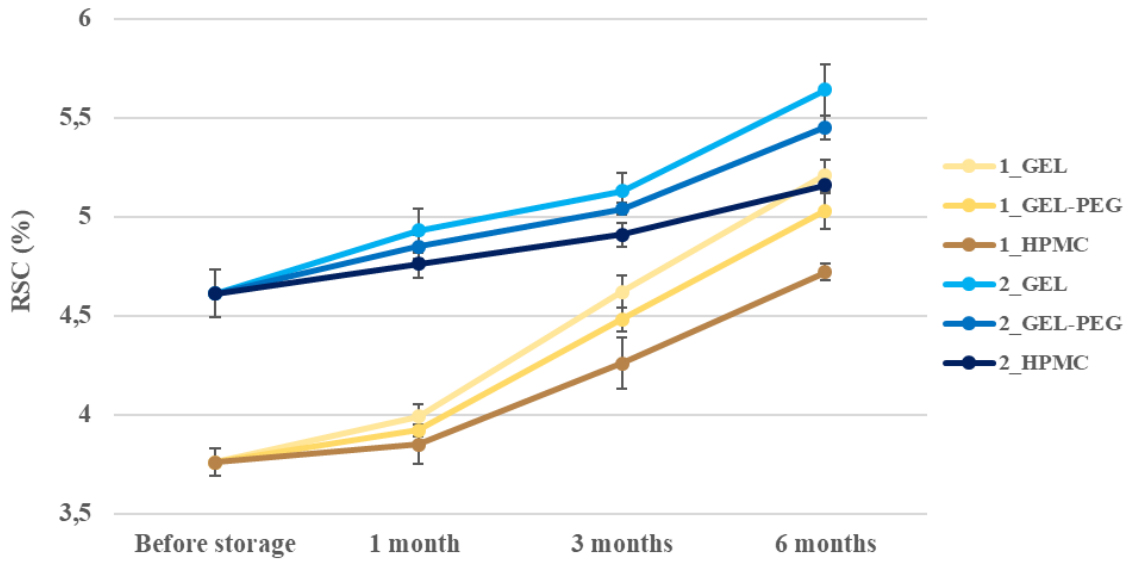


Figure 4. *RSC values of formulation (1) and (2) during the long term stability test, stored in different DPI capsule types.*

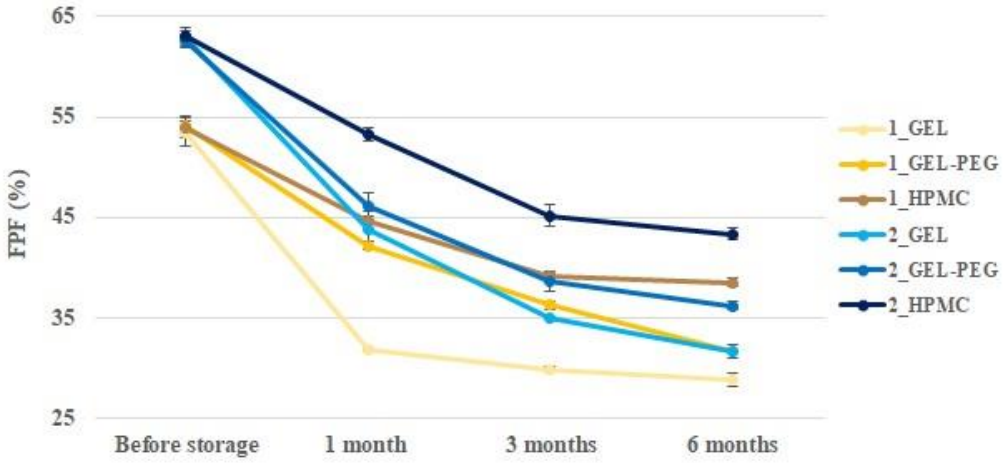


Figure 5. *FPF results at 28.3 L/min flow rate of formulation (1) and (2) during the long term stability test, stored in different DPI capsule types.*

The importance of final dosage form development was demonstrated by studying the impact of DPI capsule types on the stability and aerodynamic properties of the formulations. The same composite has different stability and thus aerodynamic properties in different DPI capsule types. The RSC (Figure 3.) and light microscope results for DPI capsules supported the claim that GEL and GEL-PEG capsules start to break when the RSC value falls below the optimum range. Due to their fragmentation, the resulting holes became irregularly shaped and large, but the deaggregation of the particles was less efficient, which in turn reduced the FPF values. The HPMC capsules, however, retained their flexibility and after 6 months, the capsule wall fragments did not break off during punching and the holes remained regular in shape. RSC and XRPD analysis and SEM images also confirmed that the particles of DPI powders stored in GEL and GEL-PEG capsules became irregularly shaped due to recrystallization (it is assumed that moisture was transferred to the DPI powders – Figure 4. –). The change in habit was least observed in composites stored in HPMC capsules, and therefore FPF values decreased to a lower extent (Figure 5.). Overall, the initial, almost identical FPF values after 6 months for HPMC capsules were the most favorable for both tested DPI formulations. The results of the novel formulated DPI after stability testing were more favorable than the carrier-free composite for all DPI capsule types. Thus, it may be worthwhile to focus on the investigation of DPI formulations in different capsules for pulmonary/DPI development, as the same formulations have different stability and thus aerodynamic properties in different DPI capsule types.

Nowadays, the study of the role of DPI capsules has been highly emphasized internationally, this work has highlighted the importance of this topic, which has already inspired other research groups to research and publish papers on this field. The presented novel formulated DPI applying CIP described may represent a new option for the therapy of lung diseases (treatment of direct and indirect pathophysiological processes in the case of CF and chronic bronchitis) instead of *per os* antibiotic products.

5. SUMMARY

In summary, the following observations can be drawn in line with the objectives of the dissertation:

I. In the literature review, we found that **CF and chronic bronchitis diseases have a similar pathophysiological process**, **which can be alleviated/slowed down by antibiotic treatment, both directly and indirectly. PDD** provides the opportunity for the antibiotic administered to act locally in lung disease, thereby **achieving a smaller dose and quick effect compared to *oral* drug administration**. Several inhaled antibiotic-containing formulations are already available on the market, typically nebulizers and DPIs, but many additional inhaled antibiotic-containing DPI formulations have been published recently. **Among the four PDD implementation options, we consider the development of DPIs to be the most promising based on their specificities**, and this was the focus of the following. The effectiveness of an inhalation powder depends on several factors:

- On the one hand, it is very important to develop a **DPI formulation** with excellent physicochemical and stability properties. We have found that there is great potential in the development of both traditional carrier-based and carrier-free DPI formulations, but formulations combining the advantageous properties of these two approaches have been published previously at a rather rudimentary, testing level.
- The second factor is the properties of the **inhalers**, including their frame design – the DPI device – and the DPI capsules used, as people with lung disease may benefit from a low-resistance inhaler due to the aerosolization and deaggregation of the particles and their aerodynamic properties, and the DPI capsules marketed may also affect the properties of the particles differently, which should be tested.
- The third influencing factor is the **patient**'s compliance/adherence and specifically how the patient uses the inhalation technique.

Based on all this literature background knowledge and previous research group experience, the **development of traditional, carrier-based and carrier-free DPI formulations containing ciprofloxacin hydrochloride** (CIP) as fluoroquinolone antibiotic **was carried out**. **From these developments, the novel formulated DPI was created as the top formulation of the Ph.D. work. For this formulation, physical investigations, *in vitro* - *in silico* testing, stability studies, and pharmaceutical dosage form development have been performed in comparison with traditional carrier-based and carrier-free formulations. The results achieved at each stage of development are detailed in the following sections.**

II. **The traditional carrier-based DPI direction has been improved** by an innovative formulation, i.e. **the application of spd CIP on MgSt surface-treated large IH carrier** ($CIP_{spd}EtOH^{10}$+IH70_MgSt). The development resulted in better *in vitro* lung deposition results than traditional carrier-based (formulated by the commercialized formulation principle) DPIs or alone, for the spd samples used in the above formulation. This development was supported by *in vitro* APSD test results for CIP and even for another API (meloxicam potassium – MXP). In fact, in both cases, **interparticle interactions** were found to correlate the physical properties of the interactive physical mixtures with *in vitro* lung deposition outcomes.

III. As a first step in the **development of carrier-free inhalation powders**, **it was studied how changing the solvent composition in the starting solution used for spd of CIP**, specifically increasing the EtOH concentration (0-30%) in addition to distilled water, affects the habit of the samples produced and thus the *in vitro* aerodynamic results. **EtOH has a major effect on the habit of the samples** due to the different evaporation rates of water and EtOH**,** mainly on the morphological properties, which also has a considerable impact on the interparticle interactions **and the *in vitro* aerodynamic behavior**. We found that the application of **30% EtOH concentration in the starting solution gave the best results** ($CIP_{spd}EtOH^{30}$). **For the first time in the literature, the terms roughness, depth and width of the dimples for particle size values were used in relation to AFM measurements, and the relationship between the trends of W_c and MMAD/D [0.5] values was also found to be novel,** which can provide new insights to explain the inhalation behavior of the samples.

As a next step in the development of **carrier-free** formulations, **co-spd CIP formulations were prepared using different concentrations of NaSt** (0-2 %). In the sample preparations, 30% EtOH was already used in the starting solutions for spd in addition to distilled water as described above. Based on the physical and *in vitro* aerodynamic results of the samples, the **co-spd formulation containing 0.5% NaSt ($CIP_{spd}EtOH^{30}NaSt^{0.5}$) was found to be optimal**.

IV. By combining the traditional carrier-based and carrier-free development results (points II-III), a novel formulated DPI formulation ($CIP_{spd}EtOH^{30}NaSt^{0.5}$+IH70_MgSt) was developed, which thus contained CIP particles co-spd with NaSt (with 30% EtOH in the starting aqueous solution) on the surface of a large IH carrier, which has been surface pre-treated with MgSt. **This composite embodies a new/third category in the preparation of DPIs besides the traditional carrier-based and carrier-free formulations, which is still new in the literature.** In line with preliminary expectations, this formulation had excellent *in vitro* aerodynamic performance of all the samples presented in the Ph.D. thesis, underlining that both carrier-based and carrier-free developments are remarkable and can be considered successful developments in their own right. ***In silico* testing of the novel formulated DPI sample as a top formulation**

was also performed, concluding that the patient can further improve lung deposition results by using the appropriate inhalation technique (10 s breath-hold time). Furthermore, **short-term room temperature stability testing** showed that after 1 month, this formulation still has favorable stability and better lung deposition results than the traditional carrier-based reference sample (CIP_{sieved}+IH70) and the carrier-free development ($CIP_{spd}EtOH^{30}NaSt^{0.5}$).

V. In the case of the **novel formulated DPI sample, a 6-month stability study was performed according to ICH guideline, testing in three different DPI capsule types**, thus developing the final dosage form of the formulation. For comparison, similar tests were also performed on the $CIP_{spd}EtOH^{30}NaSt^{0.5}$ sample. The novel formulated DPI sample also showed better lung deposition results than the carrier-free microcomposite after 6 months. Furthermore, the use of HPMC DPI capsules provided the best stability for both composites.

New findings/practical relevance of the work:

- The literature review showed that there is great potential for the development of inhalation formulations containing **antibiotics** because they **can slow down the similar pathophysiological progression in CF and chronic bronchitis in both direct and indirect ways**. Based on their advantageous specificities, we have targeted the development of DPIs for ciprofloxacin hydrochloride (CIP). **In the context of DPI systems**, it has become clear that **three pillars** (DPI formulations, DPI device and capsules, role of the patient) **need to be taken into account to maximize their effectiveness. Our development is already moving towards a therapeutic approach.**
- Within the scope of the Ph.D. work, an innovative carrier-based formulation was developed, which contains an spd CIP on the surface of a large IH carrier surface treated with MgSt by turbula mixing, whose justification for the development has been confirmed by other API (meloxicam potassium – MXP). It is a solution that goes beyond the traditional carrier-based system and exceeds the lung deposition performance of spd API, thus **providing a stepping stone for the development of other carrier-based surface treatment solutions**. The work also highlights the **importance of studying the interparticle interactions** between carrier-API and API-API particles, both to aid development and to provide an explanation for the behavior of specific formulations during inhalation.

- For drug particles developed for PDD by spd, it has been found that the starting solvent ratios used in spd can have a remarkable impact on *in vitro* lung deposition results. In the present case, using CIP, **varying the amount of EtOH** used in addition to distilled water **in the starting solution markedly affected the habit of the prepared particles, which also influenced the *in vitro* aerodynamic properties**. **The result of our work was CIP coupled solvent composition optimization. Furthermore, the Ph.D. work highlights the role of pre-experiments with initial solvent mixtures used in spd in DPI development.**
- **During the Ph.D. work, new expressions have also been created concerning AFM measurements** (roughness, depth and width of the dimples for particle size values), **which, to our knowledge, have not yet been reported in the literature.**
- **A new correlation** has been found for CIP-containing DPI samples, **MMAD/D [0.5] ratio correlates with W_c results**, which is also not yet reported in the literature. The correlation provides an explanation for the *in vitro* lung deposition results from a novel approach.
- **In the case of CIP and MXP, the preparation of NaSt co-spd carrier-free DPI formulations was carried out for the first time on an international level and resulted in successful development.**
- **The development of a novel formulated DPI** combining carrier-based and carrier-free development results / CIP containing drug particles co-spd with NaSt (with 30% EtOH in the starting aqueous solution) on the surface of a large IH carrier surface treated with MgSt /, **will launch a new category of inhalation powders. Therefore, in the future, the effective combination of traditional carrier-based and carrier-free formulations could give a new impulse to the development of DPI systems at the international level.**
- **The development of a novel formulated DPI composite dosage form has shed light on how different DPI capsule types can affect the habit, stability and *in vitro* aerodynamic performance of the formulations.**

PUBLICATIONS RELATED TO THE SUBJECT OF THE THESIS

1.) **Benke E.**, Szabóné Révész P., Hopp B., Ambrus R.: Hordozó alapú száraz porinhalációs rendszerek jellemzése és fejlesztési lehetőségei. Acta Pharmaceutica Hungarica, 87, 59-68 (2017).

Q4, IF_{2017}: -, Citations: 4 (Independent citations: -)

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Q4, IF_{2017}: -, Citations: 3 (Independent citations: -)

3.) **Benke, E.**, Farkas, Á., Balásházy, I., Szabó-Révész, P., Ambrus, R.: Szárazporinhalációs készítmények bevitelére alkalmas eszközök és modern szerelékek aktualitásai I. Gyógyszerészet/Pharmacy, 62, 131-139 (2018).

Q: -, IF_{2018}: -, Citations: 2 (Independent citations: -)

4.) **Benke, E.**, Hopp, B., Farkas, Á., Balásházy, I., Szabó-Révész, P., Ambrus, R.: Szárazporinhalációs készítmények bevitelére alkalmas eszközök és modern szerelékek aktualitásai II. Gyógyszerészet/Pharmacy, 62, 195-201 (2018).

Q: -, IF_{2018}: -, Citations: -

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Q: -, IF$_{2021}$: -, Citations: -

Σ IF: 28.032

Σ Citations: 62 (Σ Independent citations: 34)

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21.) Révész P., **Benke E.**: Terápiaspecifikus gyógyszerfejlesztés, fókuszban a krónikus tüdőbetegségek: COPD és cisztás fibrózis a transzlációs medicina tükrében. Magyar Gyógyszerésztudományi Társaság: E-learning (online), 2021.08.31.

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