

1 **Stability test of novel combined formulated dry powder inhalation system**
2 **containing antibiotic: Physical characterization and *in vitro-in silico* lung**
3 **deposition results**

4 Edit Benke^a, Árpád Farkas^b, Imre Balásházy^b, Piroska Szabó-Révész^a, Rita Ambrus^{a*}

5 ^a Institute of Pharmaceutical Technology and Regulatory Affairs, University of Szeged,
6 Szeged, Hungary

7 ^b Centre for Energy Research, Hungarian Academy of Sciences, Budapest, Hungary

8 *Corresponding author:

9 Dr. Habil. Rita Ambrus PhD

10 e-mail: arita@pharm.u-szeged.hu

11 Tel: +36-62-545-572

12

Abstract

Objective: The aim was to study the stability of dry powder inhaler (DPI) formulations containing antibiotic with different preparation ways -carrier-based, carrier-free, and novel combined formulation - and thereby to compare their physicochemical and *in vitro-in silico* aerodynamical properties before and after storage.

Significance: Presenting a novel combined technology in the field of DPI formulation including the carrier-based and carrier-free methods, *it is the most important reason* to introduce this stable formulation for the further development of DPIs.

Methods: The structure, the residual solvent content, the interparticle interactions, the particle size distribution and the morphology of the samples were studied. The aerodynamic values were determined based on the Cascade Impactor *in vitro* lung model. We tested the *in silico* behaviour of the novel combined formulated samples before and during storage.

Results: The physical measurements showed that the novel combined formulated sample was the most favourable. It was found that thanks to the formulation technique and the use of magnesium stearate have a beneficial effect on the stability compare with the carrier-based formulation without magnesium stearate and carrier-free formulations. The results of *in vitro* and *in silico* lung models were consistent with the physical results, so the highest deposition was found for the novel combined formulated sample during the storage.

Conclusion: It can be established that after the storage a novel combined formulated DPI contained amorphous drug to have around 2.5 μm mass median aerodynamic diameter and nearly 50 % fine particle fraction predicted high lung deposition *in silico* also.

Keywords: novel combined formulation, pulmonary drug delivery, ciprofloxacin hydrochloride, sodium stearate, magnesium stearate, *in silico* assessment, interparticle interactions

1. Introduction

1
2 Cystic fibrosis (CF) is an autosomal recessive hereditary disease, caused by mutations in the
3 gene that encodes the cystic fibrosis transmembrane conductance regulator (CFTR) protein
4 [1,2]. Due to the mutation, ion transports are modified through the membrane of airway
5 epithelial cells. As a result, the pH of the airway surface liquid is lowered, the mucus is
6 concentrated, mucociliary clearance efficiency is decreased, and the inflammation causes
7 mucin hypersecretion, which promotes bacterial infection [3–6]. “Polymicrobial” infection –
8 which is defined as an individual patient at a particular point of time infected with a number
9 of different organisms – is characteristic of CF. The most typical bacteria are: *Pseudomonas*
10 *aeruginosa*, *Haemophilus influenzae* and *Burkholderia cepacia* (Gram-negatives);
11 *Staphylococcus aureus* (Gram-positive). *Haemophilus influenzae* and *Staphylococcus aureus*
12 cause the early infections of CF respiratory tract, then *Pseudomonas aeruginosa* becomes the
13 most significant pathogen in adulthood [7]. In CF more effective anti-infective and anti-
14 inflammatory treatments are required to control ongoing inflammation, tissue destruction, and
15 exacerbations. Therefore the formulation of potent inhaled agents would offer significant
16 benefits for the prevention and treatment of pulmonary bacterial infections. The key
17 challenges of the therapy for airway inflammation, structural changes and mucociliary
18 dysfunction are opportunities for novel inhaled drug formulations [8,9].

19 Ciprofloxacin hydrochloride is the hydrochloride salt form of ciprofloxacin. This drug is a
20 second generation fluoroquinolone antibiotic, which is a fluorinated derivative of nalidixic
21 acid [10,11]. Ciprofloxacin is effective against both Gram-positive and Gram-negative
22 microorganisms. In point of its mechanism of action, the main target is the bacterial enzymes
23 DNA gyrase (topoisomerase II) in Gram-negative bacteria and topoisomerase IV in Gram-
24 positive bacteria [12,13]. Therefore, it may be used for respiratory bacterial infections in
25 patients with CF [14].

1 Drugs (e.g. antibiotics) can be delivered via the pulmonary route for the purpose of achieving
2 local and systemic effects. This type of drug delivery has many advantages. For example, it
3 should be noted that by circumventing the gastrointestinal tract, the drugs reach the C_{max} value
4 in the blood within approximately 1-3 minutes [15]. By avoiding the first-pass effect of the
5 liver and the enzymatic inactivation of the gastrointestinal system as metabolic pathways, the
6 use of lower doses of active agents is sufficient to induce the same therapeutic effect. Thus,
7 the side effects profile could be modified. In addition, pulmonary drug delivery is a non-
8 invasive therapeutic procedure, which does not cause pain or tissue damage [16,17].
9 However, at present only three inhaled antibiotics (tobramycin, aztreonam and colistimethate
10 (sodium)) are on the market [18]. The use of the dry powder inhalers (DPIs) offers
11 outstandingly many benefits: propellant-free, easy to use, portability, increased stability, less
12 need for patient coordination, etc. [19–21].

13 The specialized literature fundamentally separates carrier-based, and carrier-free systems
14 based on the formulation of DPI systems. Both formulations have advantages and
15 disadvantages. Most of the DPIs available on the market are made with carrier-based
16 formulation, which involves applying the active ingredient particles to the surface of a large
17 carrier particle by forming an interactive physical mixture. The use of carriers is an advantage
18 in the case of active ingredients that have a strong cohesive property, the flow properties of
19 the composition are improved, applying of the small doses of the active substance could be
20 easier by dilution with carrier, and the taste of the carrier confirms successful inhalation by
21 the patient [22–24]. However, most of these compositions do not yet have outstanding lung
22 deposition. These formulations have an average of 20-30 % fine particle fraction (FPF),
23 meaning that the drug reaches the deeper layers of the lungs in a low percentage [25]. In the
24 case of carrier-free DPI systems, the use of special excipients (e.g. L-leucine) and
25 technologies (e.g. co-spray-drying) makes the application of a large carrier avoidable.

1 Generally, these systems have low density and special morphology. However, they have
2 around 50-60 % FPF results due to the apparent high cohesive properties between the active
3 ingredient's particles [26,27]. Many publications deal with the development of DPI containing
4 ciprofloxacin or ciprofloxacin hydrochloride [12,28–33]. A serious challenge of our previous
5 work was using the benefits of these two formulations (applying 1:10 ratio and current
6 inhaled antibiotics are ~100 mg), the novel combined formulation (a co-spray-dried drug
7 blended with surface modified lactose) produced by us resulted in a higher FPF value than the
8 carrier-based and carrier-free DPI formulations [18].

9 The aim of the present work was – on the basis of the aforementioned publication [18] – the
10 stability testing of the carrier-based formulation; carrier-free formulation and novel combined
11 formulation DPI systems, which contain ciprofloxacin hydrochloride. Before and after the
12 storage we investigated the morphology, particle size and structure changes of prepared
13 formulations, as well as the modification of interparticle interactions, and mainly how these
14 physical changes influence the *in vitro* aerodynamic parameters. Furthermore, our aim was to
15 carry out computer simulations of lung deposition (from now on termed as *in silico* modeling)
16 at the stability test times with the novel combined formulated samples and compare these
17 results with the *in vitro* aerodynamic results.

18

19 **2. Materials and methods**

20 **2.1. Materials**

21 Micronized ciprofloxacin hydrochloride (μ CIP) (D50: 5.09 μ m), was kindly provided by Teva
22 Pharmaceutical Works Ltd. (Debrecen, Hungary). Lactose monohydrate, Inhalac[®] 70 (IH 70)
23 (D50: 215.00 μ m) was obtained from MEGGLE Group (Wasserburg, Germany) and used as a
24 carrier. Magnesium stearate (MgSt) (D50: 6.92 μ m) was applied as a surface modifier

1 (Sigma-Aldrich, Budapest, Hungary) of the carrier [34]. Sodium stearate (NaSt) (Alfa Aesar,
2 Heysham, United Kingdom) was used for a surface modifier of the co-spray dried particles
3 [35]. Both of them are frequently applied moisture protective agents [36,37].

4 **2.2. Methods**

5 *2.2.1. Preparation of the samples*

6 For the stability test, we again produced the samples which had been examined in our
7 previous work [18]. We prepared carrier-based, carrier-free, and novel combined formulated
8 DPI systems. Table 1. contains the w/w % compositions of these samples. The carrier-based
9 formulation (μ CIP+IH70) – as a reference [38] – was prepared with mixing in 1:10 [39] mass
10 ratio of the drug and carrier by turbula blending (Turbula System Schatz; Willy A. Bachofen
11 AG Maschinenfabrik, Basel, Switzerland) for half an hour at 60 rpm [36]. The **carrier-free**
12 formulation (CIP_0.5NaSt_spd) was produced from a solution with co-spray-drying of CIP
13 and NaSt. Firstly, we made a 1.5 w/v % aqueous solution using CIP and the alcoholic solution
14 containing 0.0175 w/v % NaSt at 30 °C. Then the two solutions were mixed in the 7: 3 ratio.
15 Büchi B-191 apparatus (Mini Spray Dryer, Büchi, Switzerland) was applied for the co-spray-
16 drying procedure with the following parameters: inlet heating temperature, 130 °C, outlet
17 heating temperature, 78 °C, aspirator capacity, 75 %, pressured air flow, 600 L/min, feed
18 pump rate, 5 %. So the solid formulation contained 99.5 w/w % of CIP and 0.5 w/w % of
19 NaSt. The novel combined formulated sample (CIP_0.5NaSt_spd+IH70_MgSt) combined the
20 two above-mentioned preparation methods supplemented with carrier surface treatment. The
21 surface modification of IH 70 carrier was made by 2.0 w/w % of MgSt (according to the
22 literature background and the applied marketed concentration [40,41]) with turbula mixing
23 for 4 h [34]. Then we prepared co-spray-dried particles as described in the carrier-free section
24 and these particles were blended with a surface smoothed carrier in the 1:10 mass ratio with a
25 turbula mixer at 60 rpm for 30 min.

1 **Table 1. Composition of the DPI formulations containing the applied concentration of**
2 **excipients.**

3 *2.2.2. Investigation of the stability of samples*

4 Stability tests were performed in Binder KBF 240 (Binder GmbH Tuttlingen, Germany)
5 equipment, with a constant-climate chamber. An electronically controlled APT.line™ line
6 preheating chamber and refrigerating system ensured temperature accuracy and
7 reproducibility of the results in the temperature range between 10 and 70 °C and the RH
8 (Relative Humidity) range between 10 and 80 %. The stability test was performed at 25 ± 2 °C
9 with 50 ± 5 % RH (room conditions). Samples were stored in hard gelatine capsules (size 3)
10 (Capsugel, Germany) in open containers; the duration of storage was 1 month. Sampling was
11 carried out after 0 and 10 days, and 1 month.

12 *2.2.3. X-ray powder diffraction (XRPD)*

13 XRPD was implemented in order to determine the crystalline form of the produced DPI
14 formulations. The powder samples were loaded in contact with a plane quartz glass sample
15 slide with an etched square, and measured with a slit detector Cu K λ_1 radiation ($\lambda = 1.5406$
16 Å) source. Settings were as follows: the samples were scanned at 40 kV and 40 mA and the
17 angular range was 3° – 40° 2θ , at a step time of 0.1 s/step and a step size of 0.01° .

18 *2.2.4. FT-IR analysis*

19 An FT-IR apparatus was used before and after storage for the study of the interaction between
20 the components and test the chemical stability of the materials. FT-IR spectra were recorded
21 with a Bio-Rad Digilab Division FTS- 65A/896 FTIR spectrometer (Bio-Rad Digilab
22 Division FTS-65A/869, Philadelphia, PA, United States) between 4000 and 400 cm^{-1} , at an
23 optical resolution of 4 cm^{-1} . Thermo Scientific GRAMS/AI Suite software (Thermo Fisher
24 Scientific Inc., Waltham, United States) was used for the spectral analysis. The sample, with

1 a CIP content of 0.5 mg, was mixed with 150 mg of dry KBr in an agate mortar, and the
2 mixture was then compressed into a disc at 10 t. Each disc was scanned 128 times at a
3 resolution of 2 cm^{-1} over the wavenumber region $4000\text{-}400\text{ cm}^{-1}$.

4 2.2.5. *Thermogravimetry (TG)*

5 Residual solvent content was investigated by TG-DTA with a Mettler Toledo TG 821e
6 thermal analysis system with the STAR^c thermal analysis program V9.1 (Mettler Inc.,
7 Schwerzenbach, Switzerland) under a constant flow of dry nitrogen gas flow of 100 mL min^{-1} .
8 Aluminium pans were applied for the samples and the reference. Scans were recorded at a
9 constant heating rate (10 °C min^{-1}) up to 350 °C . The TG-DTA oven was pre-equilibrated at
10 room temperature and each sample (ranging between 12 and 20 mg) was weighed as fast as
11 possible in order to minimize moisture uptake or release from the sample. The mass losses
12 were recorded, and the moisture contents [% wet basis] were evaluated from the normalized
13 scans, the actual mass is divided by the initial mass. The loss of water basically occurred
14 between 5 and 110 °C , and the higher temperature was used for the determination of bound
15 water.

16 2.2.6. *Interparticle interactions*

17 Contact angle (Θ) was determined by using a Dataphysics OCA 20 apparatus (Dataphysics
18 Inc. GmbH, Germany), from which we could count some of the correlations (see below). The
19 pastilles were pressed from 0.10 g of the samples with 1 ton compression force (Perkin Elmer
20 hydraulic press, Waltham, USA). Six pastilles were made of each sample. Of this, three were
21 dripped with distilled water (as a polar liquid) and the other three pastilles were dripped with
22 diiodomethane (as dispersion liquid). Thus, we obtained the contact angle of the two different
23 fluids by three parallel tests per sample. At the same time as the dropping, we made a
24 recording by using the device in 1-25 seconds time interval, so it was possible to detect and

1 determine the change of the contact angle. The surface free energy (γ_s) of the samples was
 2 calculated based on the Wu-equation. This energy consists of two parts: a disperse part (γ_s^d)
 3 and a polar part (γ_s^p), thereby ($\gamma_s = \gamma_s^d + \gamma_s^p$). The surface tension of the liquids is known in
 4 literature ($\gamma_l = \gamma_l^d + \gamma_l^p$): distilled water $\gamma^p=50.2$ mN/m, $\gamma^d=22.6$ mN/m and diiodomethane
 5 $\gamma^p=1.8$ mN/m, $\gamma^d=49$ mN/m [42]. In the Wu-equation, therefore, there are only two unknowns:
 6 the disperse (γ_s^d) and the polar component (γ_s^p) of the solids tested, which can already be
 7 expressed.

8 The Wu-equation is the following [43]:

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

9
 10 where Θ = contact angle; γ = surface free energy; s = solid phase; l = liquid phase; d =
 11 dispersion component; p = polar component

12 Cohesion work (W_c) corresponds to twice the surface free energy [44]:

$$W_c = 2 * \gamma_s$$

13
 14 The adhesion work (W_{adh}) that can be interpreted between the two different materials
 15 (represented by numbers 1 and 2) can be determined from the dispersion (γ_s^d) and polar
 16 component (γ_s^p) values calculated for the material in the present formula γ^d and γ^p , and it
 17 equals [44]:

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

18
 19 Several models are known for the determination of adhesion force (F_{adh}). In our present work
 20 we used Derjaguin's approach, which is commonly used in pharmaceutical technology [43]:

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

1

2 where R_A and R_B are the radius of the A and B particles, between which adhesive interactions
 3 were measured. R was defined as half of D [0.5], which was determined in the particle size
 4 analysis of the used raw materials.

5 The spreading coefficient (S_{12}) shows the spreadability of one material (1) on the surface of
 6 the other material (2). Conversely, it can be calculated. It is used in two-component systems
 7 to characterize distribution. This coefficient is a dimensionless number. Spreading is
 8 favorable if the result is a positive value, and the higher the number. In this case, the
 9 spreading of the drug particles can be characterized on the surface of the carrier. The
 10 coefficient or reverse case can be calculated using the following equations [43,44]:

11

$$S_{12} = 4 \left[\frac{\gamma_1^d \gamma_2^d}{\gamma_1^d + \gamma_2^d} + \frac{\gamma_1^p \gamma_2^p}{\gamma_1^p + \gamma_2^p} - \frac{\gamma_1}{2} \right]$$

12

$$S_{21} = 4 \left[\frac{\gamma_1^d \gamma_2^d}{\gamma_1^d + \gamma_2^d} + \frac{\gamma_1^p \gamma_2^p}{\gamma_1^p + \gamma_2^p} - \frac{\gamma_2}{2} \right]$$

13 where γ^d is the disperse part of surface free energy and γ^p is the polar part of surface free
 14 energy and γ is the total surface free energy of the components whose is spread on the other
 15 component.

16 2.2.7. Particle size analysis

17 The particle size distribution of the used active ingredients, excipients, and the formulations
 18 before and after storage from the dry dispersion unit were also measured by laser light
 19 scattering (Malvern Mastersizer Scirocco 2000, Malvern Instruments Ltd., Worcestershire,
 20 UK). Approximately 0.5 g of composition was loaded into a feeder tray. In the dry analysis

1 method, the air was used as the dispersion agent for the sample particles. The dispersion air
2 pressure was adjusted to 2.0 bars in order to determine whether particle attrition had occurred.
3 At least three repeated measurements were made on each sample, and the mean value was
4 calculated. Particle size distribution was characterized by the D[0.1], D[0.5], and D[0.9]
5 values.

6 *2.2.8. Scanning electron microscopy (SEM)*

7 The morphology of the samples was investigated by scanning electron microscopy – SEM –
8 (Hitachi S4700, Hitachi Scientific Ltd., Tokyo, Japan). The samples were coated with an
9 electrically conductive coating (Bio-Rad SC 502, VG Microtech, Uckfield, UK). The air
10 pressure was 1.3-13.0 MPa. In brief, the samples were sputter coated with gold–palladium (90
11 seconds) under an argon atmosphere applying a gold sputter module in a high vacuum
12 evaporator and the samples were studied using SEM set at 10-15 kV.

13 *2.2.9. Aerodynamic assessment with the Andersen Cascade Impactor Model*

14 The *in vitro* aerodynamic properties of the formulations were tested with the Andersen
15 Cascade Impactor (ACI) (Copley Scientific Ltd., Nottingham, UK), which is a most
16 commonly used to characterize the aerosolization performance of the inhaled DPIs. This
17 corresponds to the United States Pharmacopeia and Ph. Eur. 2.9.18 requirements [26,45]. The
18 vacuum pump (High-capacity Pump Model HCP5, Critical Flow Controller Model TPK,
19 Copley Scientific Ltd., Nottingham, UK) provided 28.3 L/min flow rate and a corresponding
20 ACI assembly was applied to that flow. The actual flow rate through the impactor was
21 detected with the mass flow meter (Flow Meter Model DFM 2000, Copley Scientific Ltd.,
22 Nottingham, UK). Before each test, to prevent particle bounce the ACI collection plates were
23 coated with a surfactant (Span 80 + cyclohexane solution; 1 + 99 w/w %), so repeated
24 inhalation into the cascade impactor was possible. In our experiments, the samples were

1 measured in a hard gelatin capsule (transparent, size 3, Capsugel, Germany). The drug content
2 of the formulations was detected with an UV/Vis spectrophotometer (ATI-UNICAM UV/VIS
3 Spectrophotometer, Cambridge, UK). The amounts charged into the capsules were determined
4 so that the CIP content per sample was 10 mg [12]. This mass corresponds to the tenth of the
5 CIP oral dose [27]. During our testing, Breezhaler[®] (Novartis) inhaler was used. The filled
6 capsule was placed in this inhaler and then with the help of the needles of the appliance the
7 capsule was punched with a definite movement. Because of the big amount of carrier lactose,
8 in the cases of carrier-based and novel formulations, to apply the same amount of CIP (10
9 mg), we used 2 capsules per one dose application. The DPI device, the mouthpiece, the
10 induction port, the eight plates of the impactor, and the filter were washed with distilled water
11 and the CIP concentration was quantified with an UV/Vis spectrophotometer (ATI-UNICAM
12 UV/VIS Spectrophotometer, Cambridge, UK) at 276 nm. Knowing the amount of the active
13 ingredient in the device and in the parts of the impactor, the emitted fraction (EF), fine
14 particle fraction (FPF) and mass median aerodynamic diameter (MMAD) were determined.
15 FPF expresses the fraction of particles having an aerodynamic diameter less than 5 micron,
16 these particles are likely to be deposited in the lungs. However, more and more publications
17 express the percentage of particles below 3 microns as they are most likely to reach the deep
18 lung [46,47]. MMAD is defined as the diameter of the particles deposited in the impactor for
19 which 50% w/w of particles have a lower diameter and 50% w/w have a higher diameter [48].
20 EF was expressed as the percentage of the drug found in the ACI (except the drug found in
21 the capsules and device). Only the drug concentration was determined by analytical method.
22 Therefore we can use this data by the calculation of emitted fraction.

23 *2.2.10. In silico characterization*

24 For the estimation of the amount of drug depositing in different anatomical regions of the
25 airways (upper airways, lungs), the most up-to-date version of the Stochastic Lung Model

1 (SLM) of Koblinger and Hofmann (1990) [49] was applied. Indeed, the impactor
2 measurements can demonstrate the repeatability of formulation batches and reveal the
3 aerodynamic properties (size, size distribution) of the sample. However, these data can be
4 used as predictors of airway deposition as well, with the mentioning that impactor
5 measurements cannot provide exact airway deposition values like the scintigraphic studies.
6 However, computer models validated against scintigraphic measurements (like the one
7 presented in this study) are able to estimate the deposited amount quite exactly. Deposition in
8 the extrathoracic region was calculated based on the formulas derived by Cheng (2003) [50].
9 Particles which were not filtered out by the upper airways were tracked in stochastic
10 tracheobronchial geometry. Airway lengths, diameters, bifurcation angles and gravity angles
11 were selected from statistical distributions based on the morphometric database of Raabe et al.
12 (1976) [51]. The architecture of the acinar airways relied on the data published by Haefeli-
13 Bleuer and Weibel (1988) [52]. Inertial impaction and gravitational settling were considered
14 as deposition mechanisms in both the bronchial and acinar parts of the airways. Particle size
15 distributions determined by Andersen Cascade impactor as part of this work were used as
16 inputs for the deposition simulations. In addition, the breathing parameters of a patient when
17 inhaling through Breezhaler[®] were used as modeling inputs (inhaled air volume: 1.7 L,
18 inhalation time: 3.2 s, breath-hold time after the inhalation: 5 s and 10 s, exhalation time: 3 s).
19 The breathing parameters were adopted from the work of Colthorpe et al. (2015) and
20 corresponded to a female patient with moderate COPD. The exact deposition values naturally
21 depend on the disease type and degree of severity, however, the main conclusions of the
22 present work would not be affected. The simulated high lung deposition values associated
23 with the formulation would even increase for patients with less impaired lung function. These
24 data correspond to the breathing parameter values measured by Colthorpe et al. (2013) [53].

1 This patient was selected because his/her inhalation parameter values yield an average flow
2 rate value very close to 30 L/min, which was applied in the present impactor measurements.

3 *2.2.11. Statistical analyses*

4 The statistical analyses were performed with the Social Science Statistics Online web page
5 2019. For the stability assessment using t-test calculation at 0.05 significance level and one-
6 tailed hypothesis (Social Science Statistics Online). All reported data are means \pm S.D of
7 three parallel measurements (n=3).

8

9 **3. Results and discussion**

10 *3.1. Structural characterization*

11 **Figure 1. Structural investigation of the formulations by XRPD before and after storage**

12 XRPD makes it possible to track the structural changes of the DPI samples during storage,
13 which can be analyzed if the XRPD patterns of CIP and of the used excipients are known.
14 Specifically, the characteristic of the solid state form of the active ingredient particles could
15 be very important, since the crystalline form or amorphous form could present results in
16 morphological differences and influences the interparticle interactions, thus affecting the
17 aerodynamic results. According to the XRPD diffractograms (Figure 1.A), we can determine
18 the characteristic peaks of the starting materials. These are the following: 12.8, 16.8 and 20.0
19 *2Theta degree* of IH 70; 8.23, 9.25, 19.22, 26.39 and 29.16 *2Theta degree* of CIP; 3.8, 5.5
20 *2Theta degree* of MgSt and 4.0, 6.0 *2Theta degree* of NaSt. All of these materials are
21 crystalline. We can conclude that the surface modification of IH 70 with 2 w/w% MgSt did
22 not cause any change in the XRPD pattern, thus not causing any structural change either.

1 In the case of samples (Figure 1.B) it can be concluded that CIP could be found mainly in
2 amorphous form in the CIP_0.5NaSt_spd, however the characteristic peaks of NaSt and CIP
3 (with small intensity) could be found on the curve before storage, but after 1 month complete
4 recrystallization is seen and the CIP XRPD pattern in the above figure is almost identical.
5 However, based on the peaks at 8.23, 9.25 and 26.39 *2Theta degree*, we can make statements
6 about carrier-based formulations as well. Thus for μ CIP+IH70 it can be established that the
7 initial crystalline nature of the active ingredient particles remains, and there is no change. In
8 the case of freshly prepared CIP_0.5NaSt_spd+IH70_MgSt, the active ingredient particles
9 were mainly amorphous similarly to CIP_0.5NaSt_spd, but after 1 month a substantial
10 amount of crystal structure change is not apparent on the XRPD pattern, which indicates that
11 CIP_0.5NaSt_spd+IH70_MgSt has greater structural stability relative to the latter
12 composition. Therefore the crystalline peaks correspond to IH 70.

13 According to the FT-IR analyses, the FT-IR spectra of the raw components and the prepared
14 samples before and after storage compared with each other (Figures are not presented in the
15 article). We concluded that no chemical decomposition was presumable.

16 3.2. Thermogravimetry (TG)

17 Table 2. Residual solvent content in samples.

18 The determination of thermogravimetric residual solvent content for DPIs is of key
19 importance in tracking the stability of samples. By increased residual solvent content
20 decreased stability is presumable. An increase in this value may indicate a decrease in
21 stability. Moisture sorption can cause the agglomeration of the particles; can modify
22 interparticle interactions and influence drug dispersion; de-agglomeration, which affects the
23 lung deposition results [54]. The percentages resulting from residual solvent content (Table
24 2.) from our measurements are realistic for DPIs [55]. We have found that the residual solvent

1 content has increased after 1 month for the $\mu\text{Cip} + \text{IH70}$ and CIP_0.5NaSt_spd formulations.
2 For example, it provides an explanation for the recrystallization of the latter composition. In
3 the case of the novel combined formulated DPI ($\text{CIP_0.5NaSt_spd} + \text{IH70_MgSt}$) residual
4 solvent content did not change, and it decreased slightly. The present of MgSt caused the
5 moisture resistance of the composition and this phenomenon already described in the
6 international literature [36] has been confirmed by us. It has also been found that the moisture
7 resistance of the DPI composition is improved by the use of MgSt as an excipient. The largest
8 residual solvent content change was observed for the CIP_0.5NaSt_spd formulation, in
9 contrast, there was no significant change in the novel combined formulated DPI
10 ($\text{CIP_0.5NaSt_spd} + \text{IH70_MgSt}$), which also contains CIP_0.5NaSt_spd .

11 **3.3. *Interparticle interactions***

12 **Table 3. Cohesion, adhesion values and spreading coefficient of the formulations.**

13 Interparticle interactions have already been studied in our previous work [18]. Cohesive work
14 (W_c) in the carrier-free formulations (between the drug particles), furthermore, adhesive work
15 (W_{adh}) and force (F_{adh}) in the carrier-based formulations (between drug and carrier particles)
16 are correlated with the *in vitro* lung deposition results. The studies were performed after a
17 period of 1 month storage, as shown in Table 3., the F_{adh} of $\mu\text{CIP} + \text{IH70}$ did not change, this
18 means that the active ingredient particles continue to adhere strongly to the carrier, so a low
19 FPF value is expected after 1 month, too. In the case of CIP_0.5NaSt_spd , W_c increased
20 substantially, approaching the value of fully crystalline μCIP , resulting from recrystallization
21 and residual solvent content growth that contribute to interparticle interaction change. As
22 cohesion between the active ingredient particles is increased, they can aggregate more easily.
23 For the novel combined formulated DPI ($\text{CIP_0.5NaSt_spd} + \text{IH70_MgSt}$), F_{adh} did not
24 increase greatly, still not reaching the value of adhesion of $\mu\text{CIP} + \text{IH70}$, and the spreading

1 coefficient (S_{21}) remained in the negative range left. The latter suggests that a vectored drug
2 position can still be assumed on the surface of the carrier, it is not completely covered with it.
3 All this - encountered with CIP_0.5NaSt_spd+IH70_MgSt - can be explained by the structure
4 testing and the residual solvent content experience. Thus, it is expected that the FPF value will
5 be outstanding in the *in vitro* lung deposition assay after 1 month.

6 **3.4. Particle size analysis and scanning electron microscopy (SEM)**

7 **Table 4. Morphology and particle size distribution of the formulations during the** 8 **storage.**

9 The study of particle size distribution and the morphology of the DPI samples also has great
10 importance during storage. According to existing literature, it can be said that the range of 1-5
11 microns is the optimal drug particle size for appropriate lung deposition. Particles greater than
12 5 microns are deposited in the throat and trachea with great probability and most of the
13 submicron particles are exhaled [56]. Furthermore, in terms of morphology, it can be stated
14 that spherical particles produced by spray-drying have a low contact area; homogeneous
15 particle size distribution and these result in a higher FPF than in the case of mechanically
16 micronized drugs [57]. Table 4 shows the results of SEM and laser light scattering. We can
17 conclude that the (average) diameters measured by Malvern and SEM are in correlation. We
18 focused on the active ingredient particles on SEM. The average particle size of the drug
19 particles remained in the range of 1-5 microns nevertheless, it increased for all formulations
20 during the stability test, which can somewhat reduce the lung deposition results. In the case of
21 the μ CIP+IH70 formulation, no aggregation or morphological changes can be observed after
22 1 month. After 1 month, the CIP_0.5NaSt_spd formulation shows the recrystallization and
23 aggregation of the particles, which is also indicated by XRPD; residual solvent content;
24 cohesion results and the significantly increased D [0.9] value. In contrast, there is no

1 significant morphological change which would refer to recrystallization; and there is no
2 aggregation even in SEM images in terms of the CIP_0.5NaSt_spd+IH70_MgSt formulation
3 containing the spray-dried drug particles – of the same method as the sample mentioned
4 above – on the surface modified carrier. We collected the D [0.5] values of the drug and the
5 carrier by the carrier-based formulations using the bimodal distribution curves (see table
6 below). However, D [0.1] and D [0.9] could be determined only for the formulations. We
7 concluded that the size of CIP in μ CIP+IH70 sample changed from 4.92 μ m to 5.34 μ m and
8 the size of IH70 changed from 180.03 μ m to 186.66 μ m. Furthermore, the size of CIP
9 _0.5NaSt_spd in CIP_0.5NaSt_spd+IH70_MgSt sample changed from 2.27 μ m to 2.57 μ m
10 and the size of IH70_MgSt changed from 171.12 μ m to 179.45 μ m. If we compare the change
11 in D [0.5] size of CIP_0.5NaSt_spd and of CIP _0.5NaSt_spd in
12 CIP_0.5NaSt_spd+IH70_MgSt we can see that in the combined formulation the size changing
13 was smaller than by the carrier-free sample. Therefore, in the case of the novel combined
14 formulated formulations, high FPF values are still expected in terms of *in vitro* lung
15 deposition.

16 3.5. *Aerodynamic assessment with the Andersen Cascade Impactor Model*

17 **Table 5. FPF value of microparticles before and after storage.**

18 **Table 6. EF and MMAD values of microparticles before and after storage.**

19 *In vitro* lung modeling with the Andersen Cascade Impactor results in FPF, MMAD and EF
20 (Table 5., 6.) that have been defined in the Method section. The quantities of the samples
21 were chosen after drug content determination, where the measured drug content was between
22 82 and 93% compared to the theoretical drug content. We concluded that these values didn't
23 change after the storage also. The lung deposition values (FPF) were based on the results of
24 physical examinations (XRPD, residual solvent content, interparticle interactions, morphology

1 and particle size). Thus, after 1 month of storage, the novel combined formulated DPI
2 (CIP_0.5NaSt_spd+IH70_MgSt) had the best FPF results, outstandingly high FPF <3 μm ,
3 which indicates a high deep-lung deposition (approximately three times the FPF <3 μm value
4 of $\mu\text{CIP}+\text{IH70}$ and double of CIP_0.5NaSt_spd). This is due to the fact that there is no
5 significant change in the structure and residual solvent content of this composition (in fact, the
6 latter changed favorably), thus the adhesion values did not increase substantially and its
7 morphology did not change the active ingredient particles. All this leads to a reduction in the
8 lung deposition result compared to the freshly made formulation. In contrast,
9 CIP_0.5NaSt_spd (it should be noted again that there is such an active ingredient particle in
10 the novel combined formulated formulation, and also that these particles passed down into the
11 lung in both formulations, but scattered from the carrier at the
12 CIP_0.5NaSt_spd+IH70_MgSt) recrystallized, the residual solvent content increased and
13 these led to an increase in cohesion work, its morphology became disadvantageous and
14 aggregated. Thus, FPF <3 μm and FPF <5 μm values almost fell by half after 1 month of
15 storage. For $\mu\text{CIP} + \text{IH70}$ (reference sample), it has been found that the FPF <5 μm value
16 remained about 20%, which is typical for most of the marketed formulations [26]. The
17 decrease in FPF, which is characteristic of all formulations, can be correlated with the
18 established average particle size increase of CIP_0.5NaSt_spd in the formulation. Concerning
19 MMAD, we found that the MMAD value is inversely proportional to the FPF values and only
20 CIP_0.5NaSt_spd+IH70_MgSt indicates that the particle size measured with laser light
21 scattering and the MMAD calculated with *in vitro* pulmonary modeling are also around the
22 ideal 1-5 micron range. The EF for the formulations containing the carrier ($\mu\text{CIP} + \text{IH70}$ and
23 CIP_0.5NaSt_spd+IH70_MgSt) was very high and was not considerably altered during
24 storage, however, this value of the carrier-free formulation (CIP_0.5NaSt_spd) increased,

1 presumably due to structural change (hence the morphology change), so the interparticle
2 interactions between the capsule wall and the particles were modified favourably.

3 **3.6. *In silico* assessment of particle deposition**

4 **Figure 2. *In silico* lung modeling results of the novel combined formulated DPI, SD < ±**
5 **3% (ET: extrathoracic airways, LUNG: bronchial and acinar parts, EXH: exhalation**
6 **fraction).**

7 The *in vitro* lung modeling we used is entirely suitable for comparing the aerodynamic
8 properties of the DPI formulations. At the same time, the results from the measurements with
9 Andersen Cascade Impactor are well complemented with the *in silico* lung modeling, which
10 takes into account parameters other than the above-mentioned results. As the *in vitro*
11 investigations revealed, the novel formulation is characterized by very high and nearly
12 emitted fraction value which remained nearly constant over time (Table 5). The fine particle
13 fractions remained also high after storage (Table 6). The MMAD values remained in the
14 favourable aerodynamic range regarding deposition (especially the MMAD value after 10
15 days of storage). All these characteristics predicted high lung deposition values not only of the
16 fresh sample, but also after storage. All these predictions were confirmed by the *in silico*
17 results depicted in Figure 2. In addition, the validated numerical models simulate the *in vivo*
18 conditions using real-spirometric data, so they give a more realistic picture of the behavior
19 patterns during inhalation as they take real clinical data into consideration. We can type in
20 individualized data based on age; sex; type and severity of lung disease. It should be noted,
21 however, that in the above-mentioned two pulmonary models, the expressed lung deposition
22 values have different interpretations (this is the explanation for the different percentages of
23 FPF values by *in vitro* and LUNG values by *in silico*), but it is absolutely possible to compare
24 the tendencies of the formulations and the two methods support each other. The *in silico*
25 measurements were carried out in Section 2.2.9. In our previous work, the *in vitro* and *in*

1 *silico* results of fresh samples (μ CIP + IH70; CIP_0.5NaSt_spd; CIP_0.5NaSt_spd +
2 IH70_MgSt) showed the same tendency [18]. The *in silico* results of the formulation with the
3 best *in-vitro* pulmonary deposition values (CIP_0.5NaSt_spd + IH70_MgSt) after 10 days and
4 1 month of storage is shown in Figure 2 with 5 s and 10 s as breath-hold time. The figure
5 reveals that, as predicted by the *in vitro* characterization, this formulation yielded high
6 simulated lung deposition fraction values. At the same time, the extrathoracic dose fraction
7 remained below 30% after storage (even decreased by storage). This is a significant
8 improvement compared to the other two formulations. The freshly produced CIP_0
9 .5NaSt_spd (carrier-free) had approximately 40 %, upper airway deposition, while μ CIP +
10 IH70 (carrier-based) yielded a 50 % value [18]. The exhaled dose fraction was approximately
11 20% and decreased by the increase of breath-hold time, while the extrathoracic dose fraction
12 proved to be insensitive to the length of breath-hold. Lung deposition was higher for longer
13 breath-hold indicating that the optimization of the inhalation technique can contribute to
14 further improving the pulmonary deposition of the novel combined formulated DPI and to
15 reducing the exhaled amount.

16 **Conclusion**

17 Stability tests were carried out on carrier-based, carrier-free, and novel combined formulated
18 DPI sample (CIP_0.5NaSt_spd + IH70_MgSt), containing antibiotic. After the storage, the
19 novel combined formulation presented advantageous aerodynamic results thanks to the
20 technological steps and the compositions. This sample has the most beneficial MMAD (2,5
21 μ m) and best FPF ($<5 \mu$ m; 50 %) results after 1 month, followed by the carrier-free, and the
22 worst results are shown by the carrier-based formulations (as concluded by, for example, high
23 residual solvent content, high W_{adh} and aerodynamically unfavourable morphology). From the
24 results of the physicochemical examinations, we can conclude that in the case of the novel
25 combined formulated sample (CIP_0.5NaSt_spd + IH70_MgSt), an appreciable amount of

1 crystal structure change is not apparent on the XRPD pattern, the residual solvent content was
2 slight due to the MgSt and NaSt content. As regards interparticle interactions, it can be stated
3 that the adhesion force of μ CIP + IH70 has remained high during the stability test, while in
4 the case of CIP_0.5NaSt_spd, cohesion work has increased considerably, indicating that this
5 formulation is easier to aggregate, which is also supported by electron microscopic images,
6 and the recrystallization on the images could be seen. Based on these results,
7 CIP_0.5NaSt_spd + IH70_MgSt introduced suitable stability, therefore required
8 physicochemical properties **compare with** the carrier-free formulation (where the preparation
9 of the contained drug particles was the same). However, after 1 month of storage, by the EF
10 values, a good percentage of all the three formulations was observed, The novel combined
11 formulated sample with the best *in vitro* lung deposition results was chosen for *in silico* lung
12 modeling, and it was in correlation with the *in vitro* aerodynamic results. It should be
13 emphasized that this sample had an extrathoracic dose fraction value below 30 % even after
14 one month, while the freshly produced samples from the other two samples also had worse
15 results. Finally, it can be stated that a novel combined formulated DPI formulation **with**
16 **favourable physicochemical characters after 1 month storage, resulted improved *in vitro-in***
17 ***silico* aerodynamic properties which could be the reason to get stable formulation for the**
18 **further development of DPIs.**

19 **Declaration of interest**

20 The authors report no conflicts of interest in this work.

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- 1 Development of Modern Medical Diagnostic Procedures and Therapies in a Translational
- 2 Approach: from a laboratory to a patient bed" project.
- 3

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