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Summary of PhD thesis

DEVELOPMENT OF DRY POWDER INHALATION SYSTEMS USING CIPROFLOXACIN HYDROCHLORIDE: NEW ASPECTS OF FORMULATION BY MEANS OF QUALITY BY DESIGN APPROACH

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1. INTRODUCTION

Inhaled therapy for medicinal purposes was used at least 4,000 years ago but using antibiotics in pulmonary dosage form takes back to 1948 when Abbot Laboratories developed the aero-inhaler for inhalation penicillin G powder (*Anderson et al., 2005; Sanders et al., 2007).* However large-scale therapeutic advance dates back in 1997, when tobramycin for inhalation was approved by the U.S. Food and Drug Administration (FDA) for use in patients with cystic fibrosis (*Konstan et al., 2011*). Respiratory tract infections affect people in all ages and are very common *(Adi et al., 2010; Antoniu et al., 2012).* The most common treatment for respiratory infection involves oral or parenteral administration of high doses of single or combined antibiotics which can show undesirable side effect because of high systemic bioavailability *(Pilcer et al., 2013; Hoiby et al., 2011).* The ability to deliver therapeutic agents to the site of action may allow efficient treatments of infection diseases to the respiratory tract and has many advantages over other routes (*Gelperina et al., 2005*). The excessive surface area of the lungs which contains the sufficient capillary vessels lead to a rapid absorption and the absorbed drug can directly reach to the blood circulation therefore bypass the first- pass metabolism in the liver and can be targeted by non-invasive methods *(Sung et al., 2007; Wu et al., 2014).* Therefore, the delivery of even low concentrations of antibiotics to the lung, the site of infection leads to much higher concentrations of antibiotics in the lung, while reducing systemic exposure and the risk of toxicity and yields therapeutic effects with smaller drug doses than the oral or parenteral route *(Yang et al., 2009; Cipolla et al., 2013).* The other big advantage of using pulmonary dosage form of antibiotics in treatment of chronic infections is, that is not associated with pain.

Abbreviations:

ACI - Andersen Cascade Impactor; CIP - Ciprofloxacin hydrochloride; CQA - Critical Quality Attributes; CPP - Critical Process Parameters; DSC − Differential Scanning Calorimetry; *DPI - Dry powder inhalation; ED - Emitted dose; EMA – European Medicines Agency***;** *FDA – U.S. Food and Drug Administration; FPF - Fine particle fraction; FT-IR - Fourier transform infrared spectroscopy; ICH - International Conference Harmonization; LEU - L-leucine; MMAD - Mass median aerodynamic diameter; PVA - Polyvinyl alcohol3-88; QbD - Quality by Design; QTPP - Quality Target Product Profile; RH - Relative humidity; RA - Risk Assessment; SEM - Scanning electron microscopy; SPD - Spray-dried; TPP - Target product profile; TEER – The Transepithelial Electrical Resistance; XRPD - X-ray powder diffraction*

Pulmonary dosage form of antibiotics can increase patient comfort and compliance, causing promoted treatment outcome, enhance the quality of life, shorten the hospitalization period and significantly decrease morbidity and mortality *(Littlewood et al., 2012; Greally et al., 2012).* In considering all of these advantages development of inhaled antibiotics to treat lung infection is a largely active field, with five approved products in the USA and further in the late stages of clinical progress *(Cipolla et al., 2013).*

Quality by design (QbD) is a holistic, systematic, risk, science and knowledge based method, focusing on extensive preliminary design in order to ensure the quality of medicinal products. The QbD method realizes a modern quality management thinking where the different quality requirements define the process's steps (Yu et al., 2008). In this case, risk assessment (RA) is considered to be the most accentual part, and the final goal is to ensure the predefined product quality. Its use in the early developments can help in having a time and cost-effective process, in closing the gap between the science and the industry, as well as can facilitate the innovation transfer process to introduce new drugs to market (Pallagi et al., 2015). According to the current Regulatory Science philosophy, QbD has to be one of the key elements of different pharmaceutical developments. Regulatory agencies (FDA) strongly recommend and welcome new drug applications that include QbD aspects.

DPI are formulated micronized drug particles with aerodynamic particle sizes of less than 5µ (*Islam et al., 2008*). DPI have conventionally applied a formulation of micronized drug mixed with a carrier excipient *(Healy et al., 2014).* These carrier excipients had been applied for prevention of agglutinative of particles because these small particles (1-5µm) due to high surface free energy tend to stick together. Without regard to this carrier excipient reduce the surface energy and overcome cohesive forces and adhesive forces but limit the flowability of API particles (*Hickey et al., 2003*). Moreover, DPI of antibiotics usually have large therapeutic doses (e.g. between 10 mg and 100 mg of antibiotics) thus the carrier causes difficulty in application of DPI due to the increased powder volume and the scaling down of the use of antibiotics via pulmonary dosage form *(Pilcer et al., 2013).* Around the last two decades there has been assumed a significant research on the design of carrier free system for DPI (*Healy et al., 2014*). Applying carrier free system enable the delivery of high dose antibiotics to the lungs possible by limiting the amount of excipient. Carrier free formulations can be handled by coated particles by lipids, amino acids, polymer and so on or can be applied by mechano-fusion dry coating process (*Boraey et al., 2013*).

2. AIMS OF THE WORK

As the primary step of this study, the literature background and marketed products of DPI in antibiotics was collected. Secondly the introduction of the key elements of QbD approach to design dry powder for pulmonary delivery application – was set up. Based on these preliminary data, the next objective was to develop a carrier-free co-spray-dried DPI product containing the board spectrum antibiotic ciprofloxacin (CIP). CIP can be useful for treatment of variety of pulmonary infection disease like CF. This development is a novelty as QbD approach was applied for designing the experimental part and the development holds the possibility of large scale up manufacturing in industry. The design of carrier-free microparticles ("green technology") with large dose of medicine along with minimum amounts of excipients is also a new tendency in the development of DPIs. The additives are carried out in small-scale amounts in the microparticles in order to build up greater aerodynamic performance and physicochemical stability along with least possible cytotoxicity. The main steps in our experiments were the following:

i. Pre-formulation: Identification of important factors for formulation of DPI based on carrier-free system by applying green technology based on the QbD approach. Focusing on the critical parameters, the practical development, connections and effects among the material characteristics, selected production process, investigation methods and final product properties in the design phase.
ii. Formulation: Study of different excipients (polymers, sugars and amino acid) to develop the pulmonary formulation with optimal size and superlative respirable fraction. Hence analysis the physiochemical properties of formulations. Afterwards investigation *in vitro* release of samples.
iii. Investigation of the stress and accelerated stability test of co-spray-dried products: Investigation influence of the relative humidity (RH) and temperature on the physicochemical properties and aerosolization performance of the formulations during storage.
iv. *Ex vivo*: Determination of the cytotoxicity of samples in epithelial lung cell line culture to screen the safety of formulation for pulmonary delivery along with investigation of permeability of formulation that one may to promote information on the availability in pulmonary formulations.

3. MATERIALS AND METHOD

3.1. Materials

3.1.1. Active pharmaceutical ingredient

CIP, a fluoroquinolone-type antibiotic was supplied by Teva Pharmaceutical Works Ltd. (Debrecen, Hungary). CIP is a board spectrum synthetic agent. It has potent and effective activity against a wide range of Gram-positive bacteria and against most Gram-negative microorganisms and it is often used in the treatment of inhalation anthrax and other lung infections.

3.1.2. Excipients

Polyvinyl alcohol 3-88 (PVA), a water-soluble synthetic polymer as a coating material was purchased from BASF (Cologne, Germany)(Satoh et al., 2014). The amino acid l-leucine (LEU) was obtained from Hungaropharma Ltd. (Budapest, Hungary). This amino acid can be co-spray-dried with active compounds to enhance drug aerosolization behavior.(Li et al., 2016) Hydroxypropyl beta-cyclodextrin (CD), a cyclic oligosaccharide was donated by Cyclolab Ltd.(Pitha et al., 1986) (Budapest, Hungary).

3.2. Methods

3.2.1. *QbD method*

To illustrate the relevant knowledge and information, an Ishikawa diagram was set up. The technical tool used for the RA was LeanQbD® software (QbDWorks LLC, Fremont, CA, USA). The interaction between the elements was described as “high” (H), “medium,” (M) or “low” (L). Its dynamism is presented in figures generated by the software.

3.2.2. Solubility of CIP at different pH

Solubility tests of CIP were carried out at 25°C either in a buffer solution (pH =1.2, 3.5, 5.6, 6.8 and 7.4) or in distilled water (pH =4.4). Solubility was measured by ultraviolet/visible spectroscopy (UV/VIS) spectrophotometry (ATI-UNICAM UV/VIS spectrophotometer, Cambridge, UK). The concentration was determined 24 h after filtering the saturated system.

3.2.3 Preparation of the microparticulate systems and process parameters

The significant solubility of CIP in distilled water allows its use as the solvent for spray-drying feed solution. Using 10% of ethanol in an aqueous solution is known to decrease the particle

size because of its fast evaporation during spray drying. Therefore, the feed solution was prepared by dissolving 1 gram of CIP plus different excipients at different concentrations in an aqueous solution containing 10% of ethanol. (**Table I**) According to literature background on the effects of organic solvent and additives on the habit (size and morphology) and aerosolization characteristics of DPI systems, the optimal excipient concentration is achieved as shown in **Table I.**

Table I: *Composition of the DPI products containing an optimal concentration of excipients*

No.	CIP [g]	LEU [g]	PVA [g]	CD [g]	Solvent [ml]
CIP	1	-	-	-	50
CIP_PVA	1	-	0.2	-	50
CIP_CD	1	-	-	0.9	50
CIP_LEU	1	0.4	-	-	50
CIP_LEU_PVA_CD	1	0.4	0.2	0.9	50

Spray drying is a one-step process through which it is possible to engineer and produce particles directly from solutions with a controlled technique. Hence, spray drying was considered to be the appropriate technique to produce a dry powder for inhalation. The spray-drying process was carried out using a Büchi Mini Dryer B-191 (BÜCHI Labortechnik, Flawil, Switzerland); the parameters were optimized as shown in **Table II.**

Table II: *Büchi Mini Dryer B-191 parameters for spray-drying procedure*

Inlet temperature [°C]	Outlet temperature [°C]	Feed rate [ml min^{-1}]	Aspiration air [L h^{-1}]	Aspiration rate [L min^{-1}]
130	75	5	600	0.065

The amount of dry powder yielded was determined between 65% and 70%. Generally during the spray-drying procedure nearly 30% of the sample could be lost. So our produced yield correlated with the normal sample production's habit.

3.2.4. Particle size analysis

Particle size distributions of the spray-dried powders were determined by laser scattering using Malvern (Malvern Mastersizer Scirocco 2000; Malvern Instruments Ltd., Worcestershire, UK).

Approximately 500 mg of product was loaded into the feeder tray. The dispersion air pressure was fixed to 2.0 bar to determine even if particle attrition had occurred. The particle size distribution was characterized by the D (0.1), D (0.5) and D (0.9) values and the specific surface area.

3.2.5. Scanning electron microscopy (SEM)

The morphology of CIP microparticles was investigated by scanning electron microscopy (Hitachi S4700; Hitachi Scientific Ltd., Tokyo, Japan) at 10 kV. The samples were gold-palladium coated (90 s) with a sputter coater (Bio-Rad SC 502; VG Microtech, Uckfield, UK) using an electric potential of 2.0 kV at 10 mA for 10 min. The air pressure was 1.3–13.0 mPa.

3.2.6. Fourier-transform infrared spectroscopy (FT-IR)

FT-IR spectra were recorded with an FT-IR spectrometer (Thermo Nicolet AVATAR 330; LabX Midland, ON, Canada) between 4,000 and 400 cm-1, at an optical resolution of 4 cm^{-1}. The sample was mixed with 150 mg of dry KBr in an agate mortar and the mixture was pressed to obtain self-supporting disks at 10 tons.

3.2.7. X-ray powder diffraction (XRPD)

The crystal structure of spray-dried powders was characterized using an X-ray powder diffraction BRUKER D8 Advance X-ray diffractometer (Bruker AXS GmbH, Karlsruhe, Germany). Settings were as follow: the samples were scanned at 40 kV and 40 mA and the angular range was 3° to 40° 2θ, at a step time of 0.1 s and a step size of 0.007°.

3.2.8. Differential scanning calorimetry (DSC)

The thermal response of each product was measured using a DSC (Mettler Toledo TG 821e DSC Mettler Inc., Schwerzenbach, Switzerland). Each sample was on equilibrate for 10 min at ambient temperature before being heated to 400°C at a rate of $5°C.\,min^{-1}$. Data analysis was performed using the STARe software (Mettler Toledo Mettler Inc., Schwerzenbach, Switzerland).

3.2.9. Aerodynamic particle size analysis

Aerodynamic particle size distribution was determined using a seven-stage Anderson Cascade Impactor (Copley Scientific Ltd., Nottingham, UK). The flow rate was set to 60 $L{\cdot}min^{-1}$. During the process, the aerosol moved along seven size stages according to the diameter of the particles and was then washed by methanol/phosphate buffer (60/40 v/v %) to collect the

deposited drug. All samples were investigated by UV/VIS spectrometry at 271 nm. The fine particle fraction (FPF) was established as the number of particles deposited at stage 2 and lower (#5 μm), divided by the total initial amount of the particles filled in the inhaler (10 mg). The mass median aerodynamic diameter (MMAD) was defined based on the graph as the particle size at which the line crossed the 50th percentile, indicating the particle diameter at which 50% of the aerosol particles by mass are larger and 50% are smaller. Drug-emitted dose (ED), defined as the percentage of CIP exiting the DPI, was determined by subtracting the amount of CIP remaining in the DPI from the initial mass of CIP loaded. To determine the drug content, 10 mg of the ciprofloxacin-bearing spray-dried microparticles was dissolved in methanol/phosphate buffer (60/40) and analyzed by UV spectroscopy.

3.2.10. In vitro release

To check the difference in drug release between the prepared products, 10 mL of phosphate buffer (pH 7.4, as the pH in the lung) was used to suspend an equivalent of 50 mg of CIP content in all products. After 1, 2, 3, 4, and 5 min, sample was taken out, filtered, and the concentration measured by UV spectroscopy at a maximum wavelength of 271 nm.

3.2.11. Stress and accelerated stability testing

Stability testing was carried out in a Binder KBF 240 (Binder GmbH Tuttlingen, Germany) equipment, with a constant-climate chamber. An electronically controlled APT line preheating chamber and refrigerating system ensured temperature accuracy and reproducibility of the results in the temperature range between 10 and 70 °C and the RH range between 10 and 80 %. Accelerated testing was performed at 40 ± 2 ºC with 75 ± 5 % RH. Samples were stored in hard gelatin capsules (size 3) (Capsugel, Belgium) in open containers; the duration of storage was 6 months. Sampling was carried out after 0 and 10 days, and 1, 2, 3 and 6 months.

3.2.12. Cytotoxicity testing

For cell culture A549 cells (ATCC, USA), a human immortalized alveolar type II like lung epithelial cell line, were cultured. Human endothelial cells derived from cord blood hematopoietic stem cells were cultured in endothelial medium (ECM-NG, Sciencell, Carlsbad, CA, USA) supplemented with 5% FBS, 1% endothelial cell growth supplement (ECGS, Sciencell, Carlsbad, CA, USA), 1% lipid supplement (100×, Life Technologies, USA), 550 nM hydrocortisone, 10 μM retinoic acid and 0.5% gentamycin in a humidified incubator with 5%

CO_2 at 37ºC. Cells were cultured for 4 days and monitored every 5 min until the end of experiments.

3.2.13. Permeability testing

For the permeability test lung epithelial cells were co-cultured with endothelial cells for ten days. The transepithelial electrical resistance, representing the permeability of tight junctions, was measured on the co-culture model regularly. This resistance was measured combined with STX-2 electrodes and expressed relative to the surface area of the monolayers (Ω cm^2).

4. RESULTS

4.1. QbD methodology and pre-formulation studies of DPI Products

For pre-formulation firstly an Ishikawa diagram set up including all the parameters influencing the desired DPI product containing CIP as the active agent.

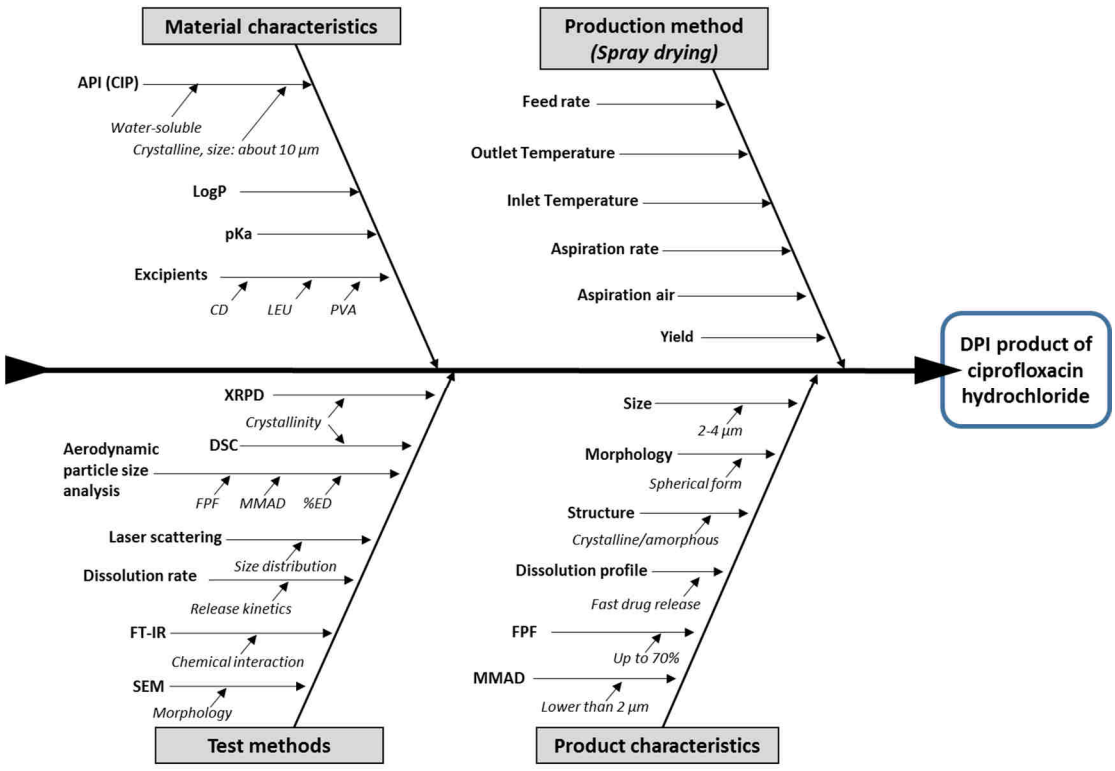

Figure 1: *Ishikawa diagram illustrating the parameters influencing the quality of the DPI product containing CIP (Karimi et al., 2016)*

The next step was the selection of the QTPPs, CQAs, and CPPs for the aimed DPI product. QbD approach was followed by risk estimation calculations using the RA software, which produced a precise impact score for each critical influencing parameter. These results of the software-based RA highlight those factors that need the highest attention during the practical development phase when we decide about the exact composition and select the materials and excipients. It has been established that the particle size of the API, the wettability and dissolution characteristics, as well as the composition of the DPI product are to be focused on during the practical development phase.

4.2. Characterization of formulated DPI containing ciprofloxacin hydrochloride

The significant solubility of CIP in distilled water allows its use as the solvent for spray-drying feed solution. Since all the powders were prepared under similar drying conditions, in the spray-drying process the final particle size distributions of the samples were comparable. The smallest particle size was measured for the DPI of CIP containing PVA. The size interval is optimal for lung deposition, so a local treatment of the respiratory tract can be achieved by any of the compositions tested (**Table III.**). From morphology aspect the DPI of CIP containing PVA has a relatively smooth surface with spherical geometry in contrast to the DPI of CIP containing LEU which was found to be cavitated. From aerosolization point the single SPD CIP formulation had a low aerosol performance (FPF=31.68±1.43). LEU has the highest potential to improve the aerosol performance (FPF=80.27%±1.65). This value indicates that more than 80% of the particles delivered from the inhaler have a volume aerodynamic diameter less than 5 µm.

***Table III**: Particle size of the microparticles of various compositions prepared*

Material	D (0.1) [µm]	D (0.5) µm	D (0.9) µm	Specific surface area [m^2/g]
CIP_SPD	1.31±0.01	2.44±0.02	4.44±0.01	2.76±0.03
CIP_PVA_SPD	1.22±0.04	2.38±0.03	4.57±0.03	2.85±0.02
CIP_CD_SPD	1.68±0.03	3.02±0.06	5.24±0.04	2.20±0.09
CIP_LEU_SPD	1.62±0.05	2.84±0.02	4.84±0.00	2.33±0.01
CIP_PVA_CD_LEU_SPD	1.58±0.06	3.27±0.01	6.42±0.02	2.14±0.02

Consequently, different DPI formulations were prepared, and all the particles produced are in acceptable particle size for optimal deposition in the lungs. Using PVA as an excipient for the CIP-containing DPI was shown to decrease the size of the microparticles significantly. The application of LEU to the microparticles induced a significant improvement in the FPF as well as the aerodynamic behavior, and allows a very fast drug release. Beside the adequate formula development, this study confirmed that all the microparticles after spray-drying are in amorphous form and therefore it was crucial to study their stability further in the process.

4.3. Physicochemical stability testing and influence of humidity and temperature on aerodynamic properties

The effects of different excipients (PVA, CD and LEU) were additionally analyzed during stability testing so as to find the appropriate excipients. In accelerated stability tests at 40 ± 2 ºC and 75 ± 5 % RH for 6 months, it was found that soluble CIP aggregation consisting of PVA and CD occurred during storage. In general, after storage median volume diameters of 20.78 ± 0.1 µm were observed for the spray-dried CIP with PVA, while values of 51.08 ± 0.1 µm were observed for samples containing CD. It means that while the polymer chains interact, they can lead to a reduction in physiochemical stabilization due to various potential interactions of the polymer chains and their ability to accept diverse conformations. Furthermore, CD can lead to an increase in particle size and the agglomeration of particles by reason of complex formation.

Aggregation was minimized in formulations with LEU due to the stabilizing effect of LEU, and the powders exhibited acceptable aerosol performance, while maintaining the FPF. The mean diameter of co-spray-dried CIP particles with LEU during storage ranged from 2.84 µm to 2.89 µm, which is suitable for targeted inhalation delivery as dry powders. The FPF value after 6 months did not decrease significantly. before storage the FPF of microparticles containing LEU was 80.27 ± 1.7 % and the MMAD value was 3.52 ± 0.13 µm. However, in this experimental work our interest was focused on retaining high aerosol dispersibility during storage. After storage, the FPF value and the MMAD value of the stored formulations was 79.78±1.22 and 2.01±0.01µm, respectively (**Table IV and V**).

Aggregation was minimized in formulations with LEU. However, the powders were slightly cohesive and unsuitable for aerosol administration. In conclusion, stability was improved by formulations with LEU. However, a balance must be achieved between the addition of enough LEU to improve physiochemical stability and prevent powder aggregation and keeping aerosol performance.

Table IV: *FPF value in % of microparticles before and after storage*

Samples	before storage	10 days	1 months	3 months	6 months
CIP_SPD	31.68±1.4	52.98±1.9	54.96±1.4	64.60±2.1	67.35±1.1
CIP_PVA_SPD	60.18±1.3	56.90±1.5	34.47±2.0	30.86±1.1	25.00±2.1
CIP_LEU_SPD	80.27±1.7	84.71±1.0	80.21±2.1	77.31±1.9	79.78±1.2
CIP_CD_SPD	58.54±1.1	47.01±1.3	40.13±1.2	38.21±1.1	36.32±1.3
CIP_PVA_CD_LEU_SPD	45.93±1.4	48.00±1.1	39.17±1.9	39.00±1.0	28.28±2.3

Table V: *MMAD value in μm of microparticles before and after storage*

Samples	before storage	10 days	1 months	3 months	6 months
CIP_SPD	7.23±0.01	4.52±0.02	4.31±0.04	3.62±0.01	3.58±0.03
CIP_PVA_SPD	3.61±0.05	4.10±0.04	7.57±0.03	8.4±0.01	11.18±0.02
CIP_LEU_SPD	2.15±0.08	2.59±0.02	2.15±0.06	2.67±0.03	2.01±0.01
CIP_CD_SPD	3.19±0.01	5.30±0.03	6.65±0.02	6.52±0.05	7.52±0.06
CIP_PVA_CD_LEU_SPD	4.53±0.02	5.47±0.05	6.55±0.03	6.98±0.02	9.11±0.07

This study presented the stable microparticles to continue the work. Accordingly, it was found that CIP aggregation consisting of PVA and CD occurred during storage. However, this aggregation was minimized in formulations with LEU regardless of presence of other excipients, due to the stabilizing effect of LEU, and the powders exhibited acceptable aerosol performance. In conclusion, stability was improved by formulations with LEU. It was shown that from five different types of microparticles three presented acceptable stability (CIP_SPD, CIP_LEU_SPD, CIP_LEU_PVA_CD_SPD). Thus, the work was proceeded with these three microparticles.

4.4. Determination of cytotoxicity and permeability of spray-dried microparticles into epithelial lung cells

From cytotoxicity view microparticles contain CIP without excipient and CIP with LEU or combinations of excipients did not change the impedance of A549 lung epithelial cell monolayers in the range of 1-300 µM concentrations, indicating no cellular toxicity. **Fig. 2** illustrates Kinetics of lung epithelial cell reaction to treatment with ciprofloxacin at 1, 10, 30, 100 and 300 µM alone or its formulations prepared with LEU, CD and PVA for 48 hours.

From Permeability aspect the transepithelial electrical resistance value of the co-culture model was 135 ± 11 Ω cm^2 measured before the permeability study and the P_{app} for the marker molecule, FD4 was 0.9 × 10^{-6} cm/s in concordance with our previous results (Walter et al., 2016). No significant difference was found in the permeability value of ciprofloxacin compared to its formulation. **Fig. 3** explains permeability of CIP (10 µM, 30 min) and its formulation on a co-culture model of lung epithelium. Values presented are means ± SEM. Statistical analysis: one-way analysis of variance followed by Bonferroni posttest. ***P < 0.001, compared to FD4 treated group, n = 4.

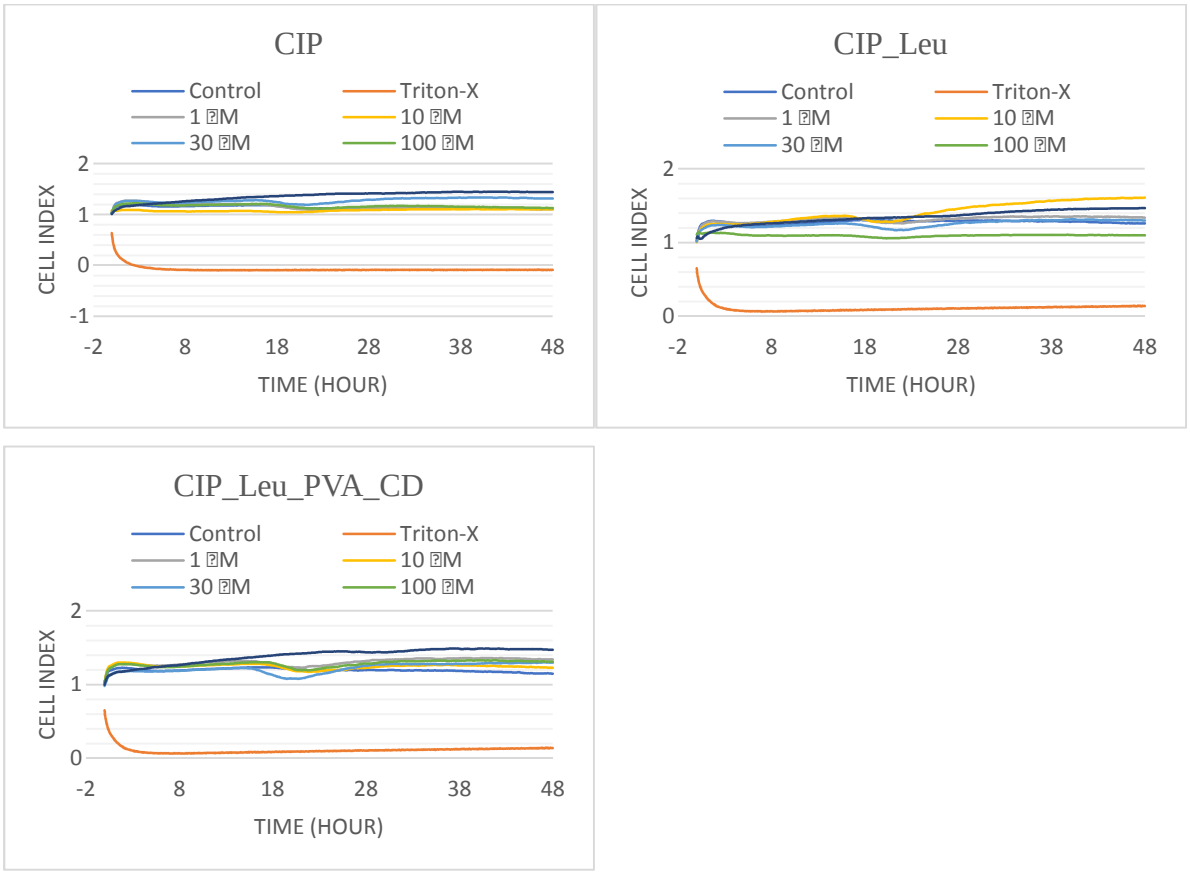

Figure 2: *Kinetics of lung epithelial cell reaction to treatment with CIP*

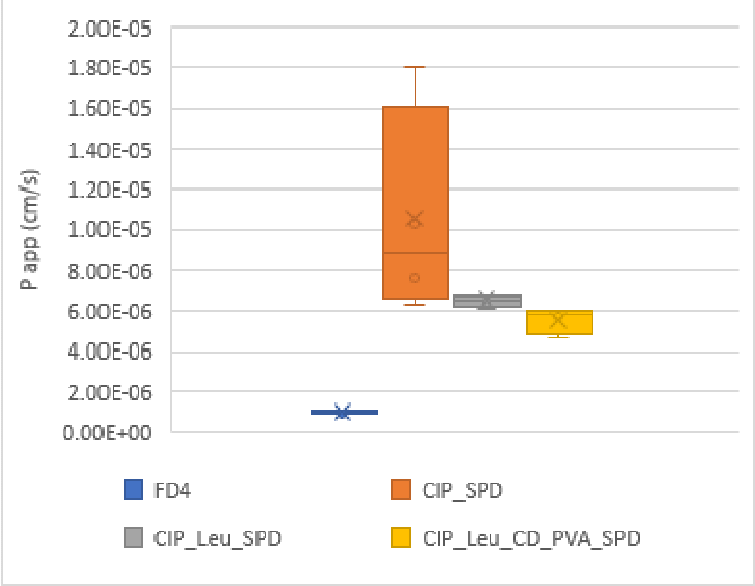

Figure 3: *permeability of CIP (10 µM, 30 min) and its formulation on a co-culture model of lung epithelium.*

5. NOVELTY AND PRACTICAL ASPECTS

This study has created the ability of development of DPI and spray-drying techniques to produce microparticles containing CIP for pulmonary drug delivery. The development of DPI of antibiotics in carrier- free system is a novelty for local treatment of respiratory tract infection.

- In this current work the application of an up-to-date and regulatory-based pharmaceutical quality management method demonstrated as a new development concept in the process of formulating DPI. This formulation produced according to the QbD methodology and Risk Assessment thinking. This innovative formulation technology and product appear to have a great potential in pulmonary drug delivery. Subsequent to no examples of QbD and RA based CIP containing DPI system formulation have been described so far.
- Co-spray-drying of CIP from an aqueous solution as innovative technology was used to prepare the novelty-type of microparticles. The advanced technology was prepared the formulation of CIP in one-step and fast process. The final microparticles which developed in green technology ensures the respirable particle size range (3-5 µm), with spherical morphology. The microparticles displayed an enhanced aerosol performance with fine particle fraction up to 80%. This high ability to aerosolization of particles is uniqueness in the DPI development.

- The formulated microparticles as innovative product tested for the stability in stress and accelerated test in long term (6 months). Since the microparticles in the dry powder system are amorphous and do not contain any stabilizer, the results of this test are very important. The stable product which may be considered suitable for scaled-up processes and pulmonary application.
- The formulated DPI illustrate a novel possibility in treatment of respiratory tract infection and the innovative technology and product present to be of great potential in pulmonary drug delivery systems.

PUBLICATIONS RELATED TO THE SUBJECT OF THE THESIS

i. Pallagi E., **Karimi K**., Ambrus R.., Szabó-Révész P., Csoka I. *New aspects of developing a dry powder inhalation formulation applying the quality-by-design approach*
International Journal of Pharmaceutics 511 (2016) 151-160

IF.: 3.649 (Q1)

ii. **Karimi K.**, Pallagi E., Szabó-Révész P., Csoka I., Ambrus R. *Development of a microparticle-based dry powder inhalation formulation of ciprofloxacin hydrochloride applying the quality by design approach*
Drug Design, Development and Therapy Journal 10 (2016) 3331-3343

IF.: 2.822 (Q1)

iii. **Karimi K.,** Katona G., Csoka I., Ambrus R. *Physicochemical stability and aerosolization performance of dry powder inhalation system containing ciprofloxacin hydrochloride*
Journal of Pharmaceutical and Biomedical Analysis 148 (2018) 73-79

IF.: 2.983 (Q1)

iv. Csoka I., **Karimi K.**, Mukhtar M., Ambrus R. *Pulmonary drug delivery: Role of antibiotic formulations for treatment of respiratory tract infection* Acta Pharm Hung (2019) *in press*

IF: - (Q4)

PRESENTATIONS

Verbal presentation

Karimi K, Szabó-Révész P, Ambrus R. *Preparation of microcomposite-based drug delivery system for DPI formulation of ciprofloxacin.* 3rd Conference on Bio-based polymers and composites. Szeged, Hungary 2016

Poster presentation

Karimi K, Ambrus R, Csoka I. *Development of antibiotic dry powder inhalation system based on Quality by Design methodology.* 12th Central European Symposium on Pharmaceutical Technology and Regulatory Affairs, Szeged, Hungary 2018.

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