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PhD thesis

DEVELOPMENT OF CO-SPRAY-DRIED CARRIER-BASED MICROCOMPOSITES FOR LOCAL PULMONARY APPLICATION OF MELOXICAM

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CONTENTS

PUBLICATIONS RELATED TO THE SUBJECT OF THE THESIS

1. **Pomázi** A., Ambrus R., Sipos P., Kása P., Szabó-Révész P.
   Formulation of Meloxicam microparticles for dry powder inhalation
   Pharmaceutical Journal of Slovenia 59 (2008) 119-121.
   IF:-

2. **Pomázi Anita,** Szabóné Révész Piroska, Ambrus Rita
   Pulmonális gyógyszerbevitel, DPI készítmények formulálásának szempontjai
   Gyógyszerészet 53 (2009) 397-404.
   IF.: -

3. Ambrus R., **Pomázi A.**, Réti-Nagy K., Fenyvesi F., Vecsernyés M., Szabó-Révész P.
   Cytotoxicity testing of carrier-based microcomposites for DPI application
   Pharmazie 66 (2011) 549-550.
   IF.: 0.869

4. **Pomázi A.**, Ambrus R., Sipos P., Szabó-Révész P.
   Analysis of co-spray-dried meloxicam-mannitol systems containing crystalline microcomposites
   Journal of Pharmaceutical Biomedical Analysis 56 (2011) 183-190.
   IF.: 2.733

5. **Pomázi A**., Buttini F, Ambrus R., Colombo P., Révész P.
   Effect of polymers for aerolisation properties of mannitol-based microcomposites containing meloxicam
   European Polymer Journal
   DOI: 10.1016/j.eurpolymj.2013.03.017
   IF: 2.739

6. **Pomázi A**, Ambrus R., Szabó-Révész P.
   Physicochemical stability and aerolization performance of mannitol-based microcomposites
   Journal of Drug Delivery Science and Technology
   accepted for publication
   IF: 1,088

OTHER PUBLICATIONS

1. Ambrus R., **Pomázi A.**, Aigner Z., Kocbek P., Kristl J. Szabóné Révész P.
   Nanotechnológia, nanokristályok a gyógyszerformulálásban
   Gyógyszerészet 52 (2008) 259-264
   IF.:-

2. **Pomázi A**., Ambrus R., Sipos P., Otomo N., Szabó-Révész P.
   Effect of a sucrose ester on co-micronized mannitol-based drug formulation
   European Journal of Pharmaceutical Sciences 38 (1) (2009) Suppl. 73-74
   IF.: 2,608

3. Ambrus R., **Pomázi A**., Szabóné-Révész P.
A nanotechnológia mint új irányvonal a hatóanyagkristályok formulálásában
Magyar Kémiai Folyóirat 3 (2010) 96-100
IF.: -

4. Ambrus R., **Pomázi A**., Kristl J., Kocbeck P., Szabó-Révész P.
Effect of high-pressure homogenization on the formulation of micro- and nanocrystals containing poorly watersoluble meloxicam
Scientia Pharmaceutica 2010. 78: 571 doi:10.3797/scipharm.cespt.8.LNM03
IF.: -

ABBREVIATIONS

ACI	Andersen Cascade Impactor
API	active pharmaceutical ingredient
AUC	area under the curve
ATTC	American Type Culture Collection
COX-2	cyclooxygenase-2
DPIs	dry powder inhalers
ED	emitted dose
FDA	US Food and Drug Administration
FPD	fine particle dose
FPF	fine particle fraction
FT-IR	Fourier transform infrared spectroscopy
GSD	geometric standard deviation
HBBS	Hank's Balanced Salt Solution
ICH	International Conference Harmonisation
LEU	L-leucine
M	mannitol
MOC	micro orifice collector
MDIs	metered dose inhalers
MDT	mean dissolution time
MMAD	mass median aerodynamic diameter
MX	meloxicam
NGI	Next Generation Impactor
NSAIDs	non-steroidal anti-inflammatory drugs
PM	physical mixture
PVA	polyvinyl alcohol 3-88
PVP	polyvinylpyrrolidone K-25
RD	recovered dose
RD 30 min	relative dissolution
RH	relativ humidity
SEM	scanning electron microscopy
SPD	spray-dried
TGA	thermogravimetry
TWEEN	Polysorbate 80
UT	ultraturrax
XRPD	X-ray powder diffraction
%DE	dissolution efficiency

1. INTRODUCTION

During recent decades, the lungs have been studied as a promising route for the administration of drugs for both local treatment and systemic therapy (Charokopos et al., 2009, Clarke et al., 1984). The action of the API in the lungs can be faster, and at the same time the possible side-effects associated with the systemic distribution are reduced. Moreover, the low metabolic activity in the lungs allows systemic delivery without liver passage and the effects of gastric stasis and pH are avoided. Further, the pulmonary route involves a large, well-perfused surface area (~100 m^2) that permits a higher absorption rate. Administration of the API directly to the lungs through the use of various aerosol delivery systems results in rapid absorption across the bronchopulmonary mucosal membranes (Weibel et al., 1963, Moren et al., 1993 Gonda et al., 2000).

The efficiency of treatment is related to the possibility that a substantial amount of API reaches the proximal airways, where it can exert its therapeutic action. This amount depends on the physiology of breathing and mucociliary clearance; the inhaler applied, and the effectiveness of the composition characteristics, such as the aerodynamic properties. As the physiological condition of the lungs varies individually, scientific studies are required to standardize the delivery systems and products (Hinds et al., 1999, Hickey et al., 2006).

Formulation of the product to be administered by the *inhalation route* is generally complicated, because it involves an active, well-defined product, and a special device. It is possible to say that the effectiveness of inhalation therapy, especially for a drug powder formulation, is dependent on factors that are related to the patient, the device and the characteristics of the formulation (Courrier et al., 2002, Chiara et al., 2008).

Among pulmonary preparations, dry powder inhalers (DPIs) can ensure stability, a high payload and patient convenience (Clarke et al., 1984). The pharmaceutics of particulate formulations are central to the performance of DPIs. The most important parameters of powder for inhalation are the particle size, particle size distribution, morphology, crystallinity of the drug and dissolution rate (Buckton 1997, Hickey et al., 2006). The DPI quality is assessed by the determination of aerodynamic properties (Wong et al., 2010) such as the aerodynamic particle size distribution, mass median aerodynamic diameter (MMAD) and fine particle fraction (FPF) (Ph. Eur. 7.2. 2012).

2. AIMS

The primary aim of this study was to establish the literature background of research and development work on DPIs. We set out to study the key factors of drugs intended for use in powder form for pulmonary delivery. Another objective was to develop a carrier-based, crystalline co-spray-dried DPI product containing the low-solubility meloxicam (MX). MX can be useful for the mono- and combination treatment of cancer, pulmonary fibrosis, inflammation and pain. The pulmonary application of MX is a novelty for local anti-inflammatory treatment because it does not exhibit aspirin-like hypersensitivity reactivity and may therefore be safely applied in therapy. The main steps in our experiments were the following:

i. the identification of important factors in the preformulation for spray-drying, preparation and characterization of mannitol-based (M) co-spray-dried samples containing MX;

ii. determination of the cytotoxicity of samples by using Calu-3 cells to screen the safe MX concentration for pulmonary delivery, in order to acquire information on the availability in pulmonary formulations;

iii. study of different adjuvants (polymers and amino acid) to optimize the pulmonary formulation properties and thereby increase the respirable fraction of co-spray-dried samples, investigation of the structure of microcomposites and aerodynamic assessment of co-spray-dried powders;

iv. investigation of the accelerated stability of M-based co-spray-dried products containing MX, and the influence of the relative humidity (RH) and temperature on the physicochemical properties and aerolisation parameters of the microcomposites during storage.

A new tendency in the development of DPIs is the design of carrier-based microcomposites with a particle size of 3-5 μm as pulmonary drug delivery systems involving different carriers and adjuvants. The additives are applied in small amounts in the microcomposites in order to promote physicochemical stability, wettability, dispersibility and aerodynamic properties.

3. LITERATURE BACKGROUND

3.1 Physiology of the respiratory tract and pulmonary deposition

The lungs are physiologically responsible for gas exchange, and the absorption of substances with anatomical and functional characteristics different from those of the organs of absorption should therefore not disturb the integrity and properties of the airway mucosa. An understanding of inhalable aerosol therapy requires knowledge of the lung function, particularly as it relates to the mechanical properties of the lungs during ventilation (Newman et al., 1983, O'Rahilly et al., 1983).

The respiratory system comprises *two functional zones*: a conducting zone and respiratory zone (**Fig. 1**).

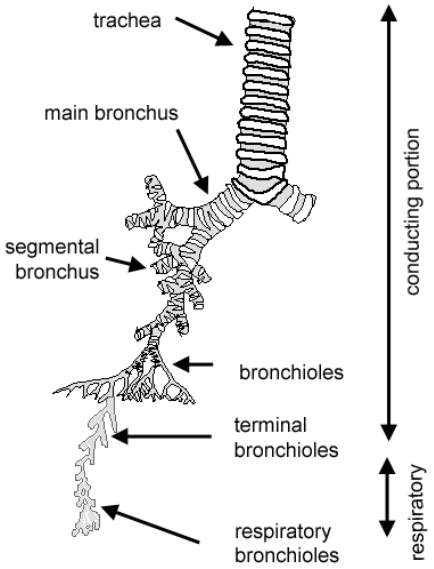


FIGURE 1: Outline of conducting airways and fragmentation, conducting and respiratory zones (http://www.histology.leeds.ac.uk/respiratory/conducting.php).

The respiratory system itself limits the entrance of extraneous particles through the geometry of the airways and the lung clearance mechanism (Lippmann 1990, Darquenne 2004). Five mechanisms control particle deposition is in the lung airways: inertial impaction, gravitational sedimentation, diffusion, interception and electrostatic attraction (Yeh et al., 1976, Stuart 1984).

These mechanisms can affect three variables: aerosol characteristics, respiratory parameters and respiratory morphologies (Gerrity et al., 1983). **Fig. 2** shows the possible mechanisms of deposition mechanism of drug particles (Charvalho et al., 2011).

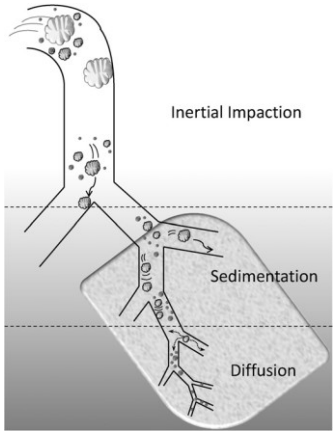


FIGURE 2: Three particle deposition mechanisms occurring within the respiratory tract (Charvalho et al., 2011).

- Inertial impaction is defined as inertial particle deposition on a surface airway. It occurs, especially near bifurcations of the airways leading to the large airways, where there is a high flow rate and rapid changes in the direction of the airflow. The flow velocities here are high and there are rapid changes in the direction of the conducting airways.
- Gravitational sedimentation takes place in the small airways, where the air speed is low and the particle size < 5 μm.
- Diffusion occurs in the small airways and alveoli where the airflow is very low and for submicrometre-sized particles (< 0.5 μm) subject to Brownian motion (Heyder et al., 1986, Patton et al., 2007).

3.2 Regional lung deposition for aerolized drugs

For particles >1 μm, deposition by diffusion is low. This means that sedimentation and impaction govern the lung deposition of inhaled drugs (Koebrich et al., 1994, Schulz et al., 2000). **Fig. 3** shows the influence of the particle diameter on regional deposition in a healthy lung in the case of slow and deep inhalation (ICRP Publication 1994). Three regions are determined: the extrathoracic region, the bronchial region and the alveolar region.

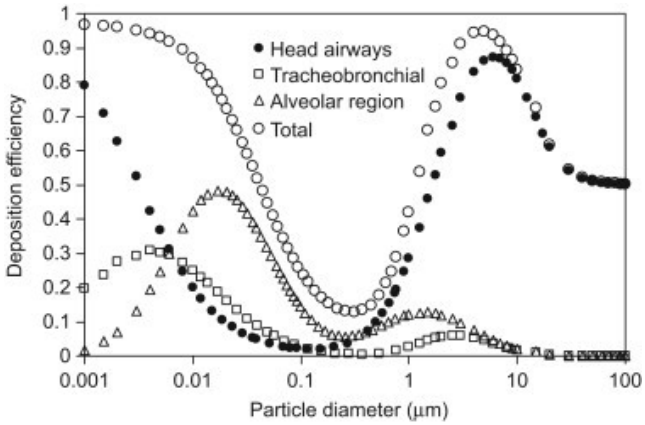


FIGURE 3: Deposition efficiencies in the respiratory tract for spherical non-hygroscopic particles of unit density on average (ICRP Publication 1994).

The total respiratory deposition exhibits a minimum at ~ 0.5 μm. At this diameter, all the deposition mechanisms are inefficient. An aerodynamic particle diameter of > 1 μm is large enough for deposition in the larger airways. At ~ 3 μm, the maximum of alveolar deposition is achieved. The reason is that these large particles are so inert, that they cannot follow the streamlines of the air into the lungs and are deposited in the mouth and throat. At a particle size >8 μm, alveolar deposition is not significant (Heyder et al., 1986).

The drug particles in the range 2-5 μm reach the intrathoracic airways, which can be considered as the therapeutic application region. The finer particles (< 2 μm) reach the alveoli, where they are absorbed by the systemic blood circulation. The advantages of this route of administration with the aim of a systemic effect include the increase in the surface to identify the alveoli, and a rich vascularization, allowing rapid absorption of the deposited drugs (Scheuch et al., 1987, Bennett et al., 1999). It prevents also degradation, which often affects orally administered drugs in gastrointestinal tract, and the metabolism of the hepatic first-pass. The aerodynamic diameter is the most appropriate parameter in terms of particle deposition by impaction and sedimentation. Diffusion depends only on the particle size, and not on the density or shape. Bronchial deposition depends on the linear dimensions of the particle, including its shape. Besides size, the profile of deposition is controlled by most other characteristics of the powder, such as the characteristics of the solid state (Brand et al., 2000).

3.3 APIs in pulmonary therapy

Inhalation drug delivery through an aerolization system is applied for both local and systemic therapy, because the alveolar epithelium is very thin (~ 0.1-0.5 μm), permitting rapid drug absorption (Patton et al., 1996).

A number of active ingredients can be delivered via the lungs for the therapy of many diseases, including respiratory and systemic therapy (**Table I**).

Table I: Active ingredients which can be delivered via the pulmonary route.

Respiratory disease	sodium cromoglycate (Aswania et al., 2002)
	formoterol, salbutamol sulfate (Becker et al., 1989)
	tiotropium (ZuWallack et al., 2004)
	beclomethasonum dipropionate, mometason furoate (Byron et al., 1994)
	itraconazole (Vaugh et al., 2007)
	ipratropium (Noord et al., 2004)
	tobramycin (Geller et al., 2007)
	indacaterol (Dahl et al., 2010)
Systemic disease	insulin (Lauble et al., 1998)
	sildenafil (Ghofrani et al., 2006)
	morphine (Mallet et al., 1997)
	vaccines (LiCalsi et al., 1999)

Non-steroidal anti-inflammatory drugs (NSAIDs) are currently the most widely prescribed medication in the world (Bianco 2000, Rabinowitz et al., 2004). NSAIDs, which have few side-effects and are not toxic to lung epithelial cells, have been proposed for pulmonary therapy. Drugs such as lysine acetylsalicylate, indomethacin and nabumeton have been investigated via the inhalation route (Tamaoki et al., 2000). MX, a cyclooxygenase-2 (COX-2) inhibitor, is a generally used NSAID. MX is practically insoluble in water. The rate of dissolution of MX can generally be improved by applying optimum formulation techniques such as the preparation of binary systems with a hydrophilic carrier, by mixing, melting or solvent methods, including particle size modification (Bashiri-Shahroodi et al., 2008, Ambrus et al., 2009, Nassab et al., 2006). Naidu et al. (2004) utilized cyclodextrins to enhance the dissolution properties of MX. This formulation possibility can be applied for the oral administration route for solid dosage forms, where a high proportion of carrier is needed to prevent the aggregation of the micronized active ingredient. Nassab et al. (2006) found that

the use of melt technology for the regular dissolution of MX resulted in mixed microcrystals. However, this technology was carried out with 10 parts of mannitol and 1 part of MX.

MX has anti-inflammatory and analgetic therapeutic effects. It is frequently used to treat rheumatoid arthritis, osteoarthritis and other joint diseases, e.g. Alzheimer's disease and cancer (Luger et al., 1996, Tsubouchi et al., 2000, Frust et al., 1997, Goldman et al., 1998). MX can be useful for the mono- and combination treatment of cancer, pulmonary fibrosis, inflammation and pain (Bednarek et al., 1999, Tsubouchi et al., 2000, Souza et al., 2005, Arafaet et al., 2007, Driessen et al., 2007). The pulmonary application of MX is a novelty for local anti-inflammatory treatment (Bavbek et al., 2007).

3.4 Delivery devices

In order to be acceptable for clinical use, an inhalation delivery system must satisfy certain qualification criteria (**Fig. 4**):

- it should generate aerosol drug carrier particles mostly < 10 microns in size (ideally, the range is 0.5 - 5 microns); the exact size depends on the designed application;
- the drug administration should be reproducible;
- the physical and chemical stability of the drug should be protected;
- the need for only minimal patient training (Chrystyn et al., 2007).

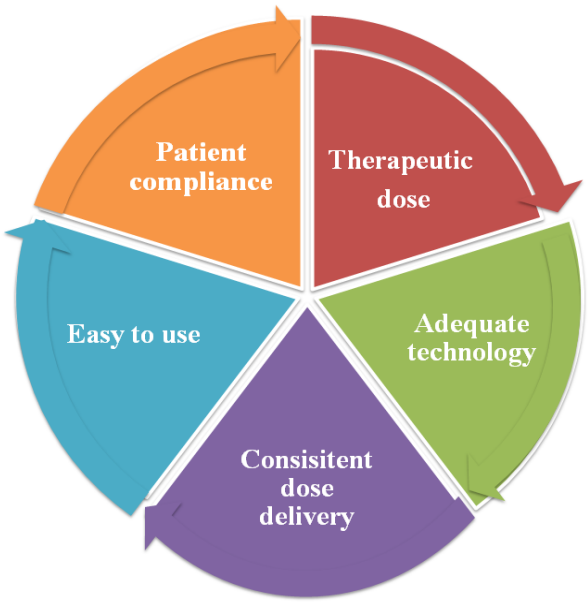


FIGURE 4: Criteria for an ideal inhaler (Chrystyn et al., 2007).

The ideal inhaler system is an easy-to-use, inexpensive, portable device that promotes patient compliance. It should protect the physical and chemical stability of the drug product. In addition, the delivery device ensures an aerosol of suitable size and reproducible drug administration. The equipment currently used to achieve the deposition of the drug suitable for pulmonary administration is of three types:

- nebulizers;
- metered dose inhalers (MDIs);
- dry powder inhalers (DPIs).

Liquid and propellant aerosol systems involve nebulizer and MDI technologies. The main difference between the two is that nebulizers use external energy to produce aerolized droplets of the formulation, while MDIs incorporate a propellant into the formulation, which provides the energy for aerolization (Newman 2005, Labiris et al., 2003). The MDI is the leading application form in inhalation therapy, because MDIs have several favourable properties, such as easy handling and high reliabilty performance. However, the traditional MDIs also have important drawbacks: difficult hand-breath coordination by the patient, and the use of environmentally damaging propellants. The MDI as a drug delivery is inefficient: only 10-15% of the dose reaches the lung (Khassawneh et al., 2008). In contrast with the MDI, the nebulizer creates mists of fine droplets with high pulmonary deposition. However, this drug delivery system utilizes compressed air, and for this reason an instrumental background is needed. On the other hand, it can take several minutes for a patient to inhale the mist from a nebulizer (Le Brun et al., 2000).

The DPIs have a number of advantages: they are propellant-free, coordination is unnecessary, and the patient's inspiratory flow is activated (Islam et al., 2008, Prime et al., 1997). Since the benefits of DPI are closely related to the inspiratory flow generated by the patient’s effort, the diseases treated by using this route of administration generally show a progression of the respiratory function and consequently the patients do not have an optimum forced expiratory volume. Numerous DPI devices are to be found on the market (Smith et al., 2003). There are essentially two types of DPIs:

- Single-dose devices

The basic mechanism is the piercing of a pharmaceutical composition-containing capsule. The inspiration of the patient generates an air flow to rotate the capsule and the dust from the air stream (Rotahaler) (Hetzel et al., 1997). The capsule is pierced in the Aerolizer (Chew et al., 1999), a device used in this study too. The Turbospin® is a breath-activated,

reusable DPI that works with a single unit capsule (containing powders), which needs to be loaded into the device each time prior to use (Islam et al., 2008).

- Multi-dose systems

These devices store the powder either in individual blisters (Diskus or Diskhaler), or in a powder reservoir (Turbohaler, Pulvinal).

3.5 Formulation for DPIs

One of the key factors involved in optimizing the DPI performance is the precision particle engineering required to produce a powder formulation that delivers accurate, consistent and effective doses of the drug.

The FDA (the US Food and Drug Administration) and the European Inhalanda group specify the requirements for the approval of new DPIs (Inhalanda 1998, FDA 1998). The US Pharmacopeia specifications for test methods harmonize with the European Pharmacopoeial requirements (European Pharmacopoeia 2012). The behaviour of particles during inhalation depends strongly on the characteristics of the product, and therefore the powder itself. One of the inherent physical properties of the final product is that it must be active, and this must be assessed. Conventional aerodynamic optimization involves a reduction in the particle size to < 5 μm. Such particles can be deposited in the respiratory tract, also size reduction methods, such as jet-milling, can influence drugs and introduce changes in the physical properties of the particles. An attractive strategy to produce particles for inhalation is to manipulate the size and density of the inhaled particles. Particles >5 μm are still able to penetrate into the alveolar region, provided that the density of the particles is such as to give a mean aerodynamic diameter of 1-5 μm, which corresponds to a density of < 0.4 g/cm^3 (Telko et al., 2005).

The bioavailability of poorly water-soluble or insoluble drugs is a well-known difficulty in the development of many pharmaceutical products. During the formulation of NSAIDs, it is strategically important to solve their solubility problems, which can lead to decrease in the drug quantity applied and to the reduction of unwanted side-effects, together with an improvement of the bioavailability (Lipinsky 2000, Hassan et al., 2004). A number of advanced technological methods are available with which to modify the physicochemical properties and increase the rate of dissolution of NSAIDs. The most common technologies are particle size reduction (Dinesh et al., 2004, Britain et al., 2002), co-crystallization (Basavoju et al., 2008), spray-drying (Paudel et al., 2013), cyclodextrin inclusion complexation (Gainotti et al., 2004), the use of inert water-soluble drug carriers in solid solutions or dispersions, the production of a suspension by a solvent evaporation method and the preparation of

nanocrystalline or amorphous forms of APIs (Brewster et al., 2007, Blagden et al., 2007, Shou-Cang et al., 2010, Oberoi et al., 2005).

Inhalation drug products are still at the focus of drug delivery, especially because the pulmonary delivery of drugs is recognized as an important delivery route for both locally and systemically acting drug substances. One of the most important issues during drug development is the choice of a suitable formulation approach and suitable excipients (Pilcer et al., 2010, Traini et al., 2012). During DPI formulation, various excipients can be applied: lipids such as dipalmitoylphosphatidylcholine can be used for the formulation of liposomes, the most extensively investigated systems for controlled delivery (Possmayer et al., 2001, Sanna et al., 2004, Desay et al., 2003). Beside lipids, several alternative excipients have been tested. Amino acids such as leucine (LEU), glycine and alanine have been shown to decrease the hygroscopicity and improve the surface activity and charge density of particles, and therefore to improve the *in vitro* and *in vivo* deposition significantly (Li et al., 2005, Seville et al., 2007, Rabanni et al., 2004, Chew et al., 1999, Seville et al., 2007). Surfactants such as Polysorbates are a new approach for low-density particle formation (Steckel et al., 2004). Chitosan, consisting of cationic polysaccharides, has been used as an absorbtion enhancer for proteins and peptides, besides its bioadhesive effect (Amidi et al., 2008, Soyele 2008). Polymers are used to engineer drug microparticles; they have influence on the properties of a microparticle by adsorbing to its surface during spray-drying. Polyvinyl alcohol (PVA) (Buttini et al., 2008) and polyvinylpyrrolidone (PVP) (Robinson et al., 1990, Pilcer et al., 2010) have the ability to adsorb at interfaces, and may therefore be used as microfine coating materials and stabilizers in DPI systems.

Different carriers (e.g. microcrystalline cellulose, lactose monohydrate, M, sorbitol, cyclodextrin, xylitol, glucose, raffinose and trehalose) are used to ensure the distribution of drugs (Tee et al., 2000, Mao et al., 2004, Steckel et al., 2004; Glover et al., 2008). They have an established safety and stability profiles; they are produced in different manufacturing processes with tight controls over purity and physical properties, they are readily available in different grades and they are inexpensive. They are advantageous as highly water-soluble compounds with low toxicity, low hygroscopicity and significant stability in the DPI formulation.

Different production techniques may be applied to obtain micronized powder suitable for pulmonary delivery. These techniques include classical ones, such as jet milling, and other more advanced methods of considerable interest for many applications, not only for medicines, such as spray-drying and spray-freeze-drying and extraction with supercritical

fluid (Chow et al., 2007, Veinhard et al., 2008, Zijlstr et al., 2004). The use of these advanced techniques of particle engineering to achieve optimization of the fine particle fraction has led to improved possibilities of aerosol formation and the drug dissolution. The optimum is based on the integration of an efficient DPI device with a powder formulation. Two powder formulation routes are commonly employed in the majority of DPIs: carrier-based systems and agglomerated systems.

In the conventional carrier-based techniques, the drug particles are much smaller than the carrier particles; the smaller drug particles preferentially adhere to the carrier, resulting in an adhesive mix (Kaerger et al., 2006). During inhalation, the energy imparted by the patient must overcome the adhesive bond formation, so that the drug particles can be liberated from the carrier to penetrate into the respiratory tract. However, the force of adhesion between the drug and the carrier may be greater than the energy supplied, and the drug will then remain on the carrier, to be swallowed, after impaction in the throat, instead of being inhaled (Young et al., 2003) (**Fig. 5**).

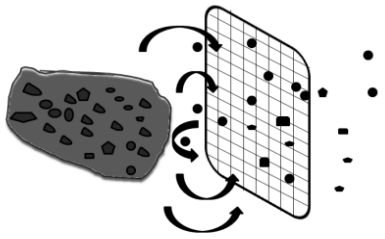


FIGURE 5: Outline of a DPI carrier-drug system.

In comparison with carrier-based formulations, agglomerate-based formulations (**Fig. 6**) do not contain large inert material particles, but re-spheronized aggregates of suitable size for inhalation. Agglomerate systems rely on the inspirational energy being sufficient to break down the powder network, resulting in primary particles of respiratory size (Wetterlin et al., 1988, Le et al., 2012).

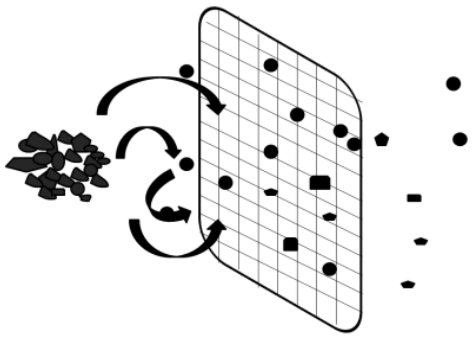


FIGURE 6: Agglomerate formation and dispersion.

Fig. 7 illustrates a novel possibility of formulation for DPIs: the development of co-spray-dried particles, where the active ingredient, the carrier and the applied additives are located in one micronized particle. A new tendency in the development of DPIs is the design of carrier-based microcomposites with a particle size of 3-5 μm as pulmonary drug delivery systems involving different carriers and adjuvants. The additives are applied in small amounts in the microcomposites in order to promote physicochemical stability, wettability, dispersibility and aerodynamic properties.

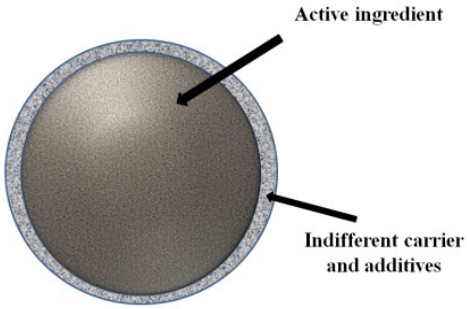


FIGURE 7: Structure of novel co-spray-dried particle.

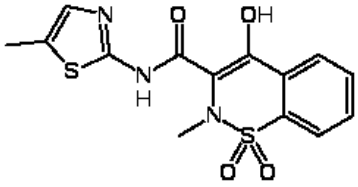
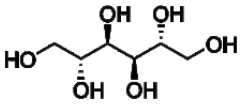
Several studies have been reported on the co-spray-drying technique with or without a carrier from aqueous solution, but spray-drying is not limited to aqueous solutions. The spray-drying of ethanolic solutions containing ipratropium bromide, salbutamol sulfate and pure beclomethason dipropionate has also have been achieved (Woolfe et al., 2001, Sakagami et al., 2002). The novel approach presented in our work was an assessment of the suitability of the co-spray-drying of MX from an aqueous **microsuspension**. The final, crystalline microcomposite was prepared in a one-step process, which additionally ensures favourable particle size, morphology and crystallinity properties.

4. MATERIALS AND METHODS

4.1 Materials

MX, a DPI-active pharmaceutical ingredient, was obtained from EGIS Ltd. (Hungary) (**Table II**).

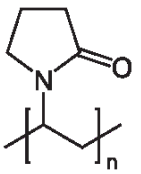
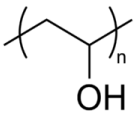
Table II: Properties of the active agent and the indifferent carrier, M.

	Meloxicam (MX)	**β-D-Mannitol (M)**
Chemical structure		
Chemical name	4-hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-2*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide	(2*R*,3*R*,4*R*,5*R*)-hexane-1,2,3,4,5,6-hexol
Physical properties	a yellow powder particle size: 85.39 ± 6.63 μm poor solubility in water	a white powder crystalline water-soluble
Applications	a NSAID, a selective COX-2 inhibitor	an indifferent carrier

M, a hydrophilic carrier, was obtained from Hungaropharma (Hungary) (**Table II**). M is a sugar alcohol, and is used as a dry powder for inhalation for the diagnosis of bronchial hyperresponsiveness (Anderson et al., 1997, Brannan et al., 2005). As an osmotic agent, M is also administered to enhance the clearance of mucus in cases of bronchiectasis and cystic fibrosis. M was primarily chosen as a model carrier in this study because its particle size can be readily controlled by spray-drying (Chew et al., 1999) and the spray dried M has a crystalline state (Rowe et al., 2001). M is highly water-soluble compound with low toxicity, low hygroscopicity and significant stability by the DPI formulation and gives an obvious sweet aftertaste (Adi et al., 2010; Zajca et al., 2005, Brauna et al., 2009). Furthermore, M is a suitable carrier for the aerosol delivery of proteins and significant stability in DPI formulations (Kaialy et al., 2012, Kaialy et al., 2013 a-b, Nokhodchi et al., 2013).

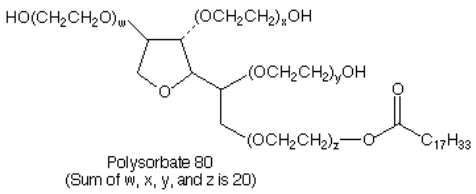
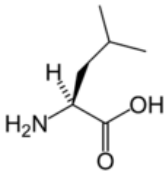
The polymer additives, polyvinylpyrrolidone K-25 (PVP) and polyvinyl alcohol 3-88 (PVA) were purchased from BASF (Germany) and from ISP Customer Service GmBH (Germany), respectively (**Table III**).

Table III: Polymer additives and surfactant in the co-spray-drying process.

	Polyvinylpyrrolidone K-25 (PVP)	**Polyvinyl alcohol 3-88 (PVA)**
Chemical structure		
Synonyms	Povidone, Polyvidone, Kollidon	Mowiol
Physical properties	a white to light-yellow powder amorphous	a white powder semicrystalline
Applications	a stabilizing agent	a stabilizing agent a microfine coating material

Tween 80 (BASF, Germany) (**Table IV**) was of pharmaceutical grade. Amino acids such as L-leucine (LEU) (Applichem, Germany) (**Table IV**) can be co-spray-dried with certain active compounds to modify drug aerolization behaviour.

Table IV: Properties and structure of the surfactant and dispersity enhancer.

	Polysorbate 80 (TWEEN)	**L-Leucine (LEU)**
Chemical structure		
Synonyms	Tween 80	2-amino-4-methylpentanoic acid
Physical properties	a viscous yellow liquid	a white crystalline powder
Applications	a non-ionic surfactant a wetting agent	a dispersity enhancer

4.2 Methods

4.2.1 Preformulation of co-spray-dried products containing MX

The compositions of samples are presented in **Table V**. The mass of each sample was 100 g. The MX content, if present, was 1 g.

Table V: Compositions of samples.

Sample	MX (g)	M (g)	TWEEN (g)	PVP (g)
MX	1	-	-	-
MX spd	1	-	-	-
M	-	1	-	-
M spd	-	1	-	-
MX-M	1	1	-	-
MX-M-TWEEN	1	1	0.1	-
MX-M-PVP	1	1	-	0.05
MX-M-PVP-TWEEN	1	1	0.1	0.05

Fig. 8 shows the preparation protocol for the carrier-based co-spray-dried systems. The particle size and form of the MX microcrystals were modified by top-down technology, which is a disintegration method involving the use of stress. The resulting microsuspensions contained the API in homogeneous distribution. From the suspensions of the drug and carrier, solid powders were obtained by spray-drying; a standard microsize was achieved.

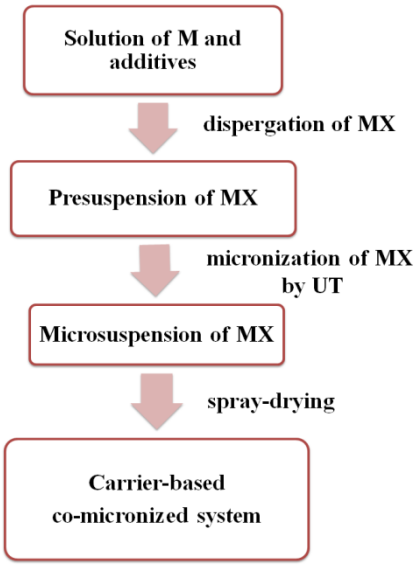


FIGURE 8: Preparation protocol for carrier-based systems.

A microsuspension was prepared from 1 g of each of MX and M, 0.1 g of TWEEN and/or 0.05 g of PVP ad 20 g with water, using an Ultraturrax (UT) (T-25 IKA-WERKE, Germany) at 6500 and 24000 rpm for 10 min, and for spray-drying a Büchi Mini Dryer B-191 (Switzerland) with 130 °C inlet and 70 °C outlet temperature; the aspirator capacity was 60% and the aspirator pressure was 25 mbar. Spray-dried MX (MX spd) and M (M spd) were prepared as controls from 1 g of MX or M ad 20 g with water, using the same preparation parameters as for the products (**Table V**).

4.2.2 Optimization of the microcomposites and the process parameters for the aerodynamic assessment of powders

The components of the microcomposites are presented in **Table VI**.

Table VI: Compositions of the products.

Product	MX (g)	M (g)	PVP (g)	PVA (g)	TWEEN (g)	LEU (g)
MX-M	5	5	-	-	-	-
MX-M-PVP	5	5	0.025	-	-	-
MX-M-PVP-LEU	5	5	0.025	-	-	0.2
MX-M-TWEEN	5	5	-	-	0.05	-
MX-M-TWEEN-LEU	5	5	-	-	0.05	0.2
MX-M-PVA	5	5	-	0.1	-	-
MX-M-PVA-LEU	5	5	-	0.1	-	0.2

Fig. 9 shows the process of formulation of the samples. The preparation parameters and compositions were optimized.

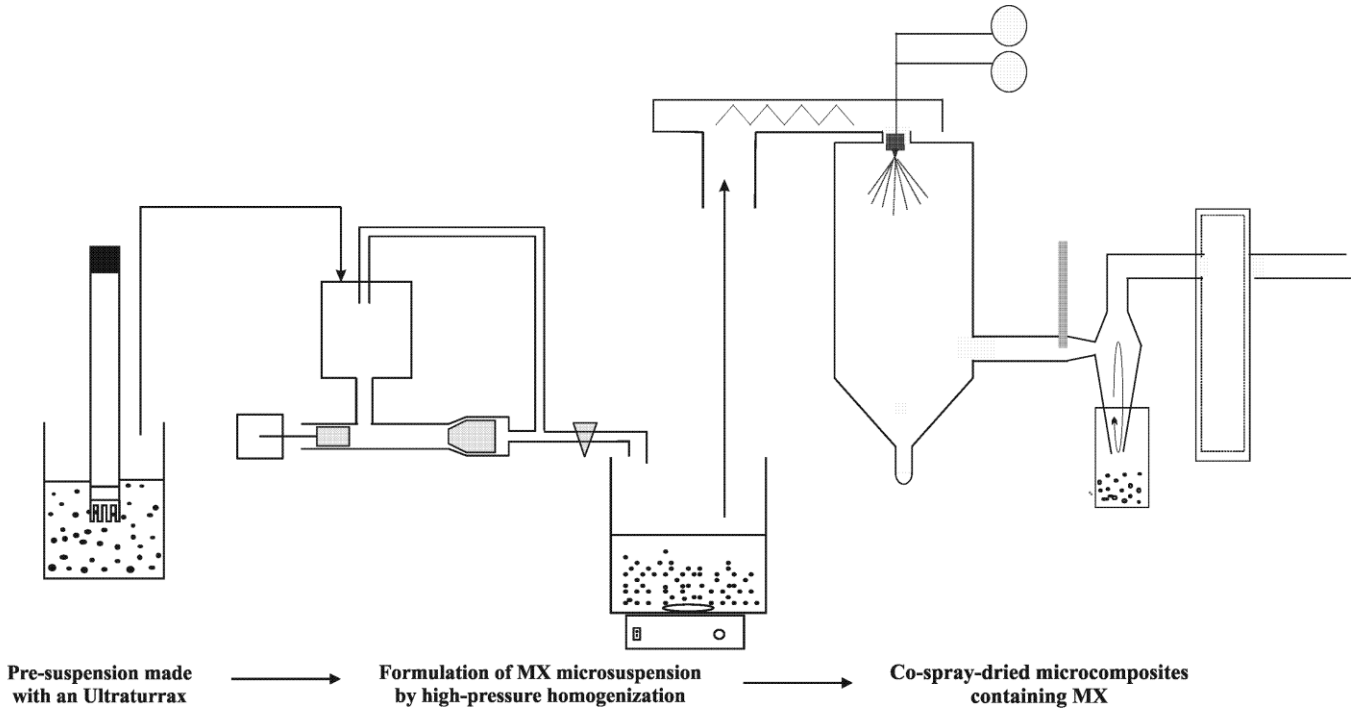


FIGURE 9: Preparation of co-spray-dried microcomposites.

Each pre-suspension was prepared from MX and M, together with TWEEN, PVP or PVA, and with LEU, and made up to 100 g with water, using an Ultraturrax operated at 24 000 rpm for 10 min. The particle size of the MX in the pre-suspension was decreased by cavitation with a high-pressure homogenizator at 1500 bar for 10 cycles. The process resulted in a microsuspension of MX which contained M, PVP or PVA and LEU in dissolved form. Such microsuspensions were spray-dried with a Büchi Mini Dryer B-191. The suspensions were homogenized with a magnetic stirrer under a drying process.

These parameters ensured optimum drying efficiency in the case of co-spray-dried samples. The spray-drying efficiency was in all cases 75-80%. 80% of the powder was situated in the sample container. During the co-spray-drying, the final, solid products were obtained, comprising microcomposites containing MX crystals in micronized form. **Table VII** indicates the spray-drying parameters.

Table VII: Spray drying conditions for co-spray drying powders.

Inlet temperature (ºC)	Outlet temperature (ºC)	Feed rate (ml min^{-1})	Aspiration air (l h^{-1})	Aspiration rate (m^3 min^{-1})
130-135	77-81	4.0-4.5	600	0.065

4.2.3 Drug content analysis

The MX contents in the spray-dried samples were determined by dissolving 6 mg of sample in 100 ml of methanol/phosphate buffer solution (60/40 v/v). The solutions were sonicated for 10 min, then diluted (1:2) and analysed spectrophotometrically (ATI-UNICAM UV/VIS Spectrophotometer, Cambridge, UK) at 362 nm. Three samples were analysed for each batch, with random sampling of the powder.

4.2.4 Cytotoxicity testing

The Calu-3 cell line was obtained from the American Type Culture Collection (ATCC, USA). Cells were maintained with regular passage in Dulbecco's Modified Eagle's Medium (DMEM, Sigma-Aldrich Ltd.) supplemented with heat-inactivated foetal bovine serum (10% v/v), sodium pyruvate 100x (1% v/v), penicillin/ streptomycin 100x (1% v/v) and non-essential amino acids 100x (1% v/v) at 37 °C in an incubator containing 5% CO_2.

For the cytotoxicity tests, Calu-3 cells were seeded in 96-well plates at a density of 10^4 cells/well. The confluent monolayers were used for cytotoxicity studies 7 days after the initial seeding. The MTT method (Mosmann 1983) was used to determine the cytotoxicity of the different formulations as follows. The formulations were dispersed in Hank's Balanced Salt Solution (HBSS) and the cells were exposed to increasing concentrations at 37 °C for 1 hour. Controls were processed identically and incubated in HBSS simultaneously. After 1 hour of incubation, cells were washed with HBSS and the MTT solution was added to each well at a final concentration of 0.5 mg/ml. After 3 hours of incubation at 37 °C, the derived formazan crystals were dissolved in isopropanol/1 N HCl (25:1) and the absorbance was measured at 570 nm with a FLUOstar OPTIMA microplate reader (BMG LABTECH, Offenburg Germany). Absorbance values were corrected for the background absorbance, measured at 690 nm. Cell viability was expressed as a percentage of the untreated control.

4.2.5 Particle size determination

The particle sizes of the solid samples were determined with a Leica Q500MC Image Processing and Analysis System (Leica Cambridge Ltd., UK). The particle size distribution of MX in the microsuspensions was analysed before spray-drying in all cases, and the particle size analysis of dry M-based systems was also carried out after co-spray-drying. The particle size of MX in the microsuspensions was analysed before spray-drying with the Malvern apparatus (Malvern Hydro 2000, Malvern Instruments Ltd., Worcestershire, UK). The volume particle size distribution was measured by laser diffraction (Mastersizer S, Malvern

Instruments Ltd., Worcestershire, UK) with the following parameters: 300RF lens; small volume dispersion unit (1000 rpm); true density of MX = 1.565 g cm^{-3} (AccuPyc 1330, Micromeritics, Norcross, USA); 1.596 was taken as the refractive index for dispersed particles, and 1.330 for the dispersion medium. The particle size distribution of the microcomposites from the dry dispersion unit was also estimated by laser diffraction (Malvern Mastersizer Scirocco 2000, Malvern Instruments Ltd., Worcestershire, UK). In the dry analysis method, air was used as the dispersion agent for the microcomposite particles from the inlet to the sample cell. Approximately 2 g of product was loaded into a feeding tray. The dispersion air pressure was adjusted to 2.0 bar, in order to determine whether particle attrition had occurred. Obscuration of between 10.0% and 13.0% was achieved throughout the entire measurement duration. The particle size distribution was characterized by the D(0.1), D(0.5) and D(0.9) values and the Span values were calculated according to Eq. 1. A high Span value (> 1) denotes a broad particle size distribution. The higher Span value, the broader the particle size distribution (Li et al., 2004).

$$Span = \frac{D(0.9) - D(0.1)}{D(0.5)} \quad (1)$$

4.2.6 Scanning electron microscopy (SEM)

The morphology of the particles was examined by SEM (Hitachi S4700, Hitachi Scientific Ltd., Tokyo, Japan). A sputter coating apparatus (Bio-Rad SC 502, VG Microtech, Uckfield, UK) was applied to induce electric conductivity on the surface of the samples. The air pressure was 1.3-13.0 mPa.

4.2.7 Fourier transform infrared spectroscopy (FT-IR)

For study of the interaction between the components in the microcomposites, an FT-IR apparatus was used before and after storage. FT-IR spectra were recorded with a Bio-Rad Digilab Division FTS- 65A/896 FTIR spectrometer (Bio-Rad Digilab Division FTS-65A/869, Philadelphia, USA) between 4000 and 400 cm^{-1}, at an optical resolution of 4 cm^{-1}. Thermo Scientific GRAMS/AI Suite software (Thermo Fisher Sciencific Inc., Waltham, USA) was used for the spectral analysis. The sample, with an MX content of 0.5 mg, was mixed with 150 mg of dry KBr in an agate mortar, and the mixture was then compressed into a disc at 10 tons. Each disc was scanned 64 times at a resolution of 2 cm^{-1} over the wavenumber region 4000-400 cm^{-1}.

4.2.8 X-ray powder diffraction (XRPD)

XRPD spectra were recorded with a BRUKER D8 Advance X-ray diffractometer (Bruker AXS GmbH, Karlsruhe, Germany) system with Cu Kα1 radiation (λ = 1.5406 Å) over the interval 5-30°/2. The measurement conditions were as follows: target, Cu; filter, Ni; voltage, 40 kV; current, 40 mA; time constant, 0.1 s; angular step 0.010°. In the determination of the degree of crystallinity, the total area of the three peaks with largest intensity was examined, after smoothing and background removal.

4.2.9 Water content determination by thermogravimetry (TGA)

Residual water content was analysed by TG-DTA with a Mettler Toledo TG 821^e thermal analysis system with the STAR^e thermal analysis program V9.1 (Mettler Inc., Schwerzenbach, Switzerland) under a constant flow of dry nitrogen gas flow of 100 ml min^{-1}. 100-µl alumina crucibles were used for the samples and the reference. Scans were recorded at constant heating rate (5 °C min^{-1}) up to 300 °C. The TG–DTA oven was pre-equilibrated at room temperature and each sample (ranging between 12 and 20 mg) was weighed as fast as possible in order to minimize moisture uptake or release from the sample. The mass losses were recorded, and the moisture contents [% wet basis] were estimated from the normalized scans, the actual mass being divided by the initial mass.

4.2.10 Contact angle

The OCA Contact Angle System (Dataphysics OCA 20, Dataphysics Inc., GmbH, Germany) was used for studies of the wettability of the carrier systems, and products containing MX. 0.15 g of powder was compressed under a pressure of 1 ton by a Specac hydraulic press (Specac Inc., USA). The wetting angles of the pressings were determined after 4.3 µl of distilled water had been dropped onto the surface of the pressings. The change in the wetting angle was registered from 1 to 25 s (a minimum of 5 parallel numbers), using the circle fitting method of the OCA System. The method of Wu was applied, in which two liquids with known polar (γ_l^p) and dispersion (γ_l^d) components are used for measurement. The solid surface free energy is the sum of the polar (γ^p) and non-polar (γ^d) components, and is calculated via the following equation (2) (Wu 1971):

$$(1+\cos\theta)\gamma_l = \frac{\left(\gamma_s^d \gamma_l^d\right)}{\gamma_s^d + \gamma_l^d} + \frac{\left(\gamma_s^p \gamma_l^p\right)}{\gamma_s^p + \gamma_l^p} \quad (2)$$

where Θ is the contact angle, γ_s is the solid surface free energy and γ_l is the liquid surface tension. The percentage polarity can be calculated from the γ^p and γ values: (γ^p /γ)*100. The liquids used for our contact angle measurements were bidistilled water (γ^p = 50.2 mN m^{-1}, γ^d = 22.6 mN m^{-1}) and diidomethane (γ^p = 1.8 mN m^{-1}, γ^d = 49 mN m^{-1}).

4.2.11 *In vitro* release

Modified paddle method

The paddle method with the USP dissolution apparatus (USP dissolution apparatus, type II Pharma Test, Heinburg, Germany) was used to examine MX and the products. The medium was 100 ml of phosphate buffer of pH 7.4 ± 0.1. The basket was rotated at 100 rpm and sampling was performed up to 120 min. After filtration (filtration pore size 0.45 µm; applying a Millex-HV syringe-driven filter unit, Millipore Corporation, Bedford, USA) and dilution, the MX contents of the samples were determined by spectrophotometry (ATI-UNICAM UV/VIS Spectrophotometer, Cambridge, UK) at 362 nm.

Statistical analysis of MX dissolution profile

The percentage dissolution efficiency (%DE) for each sample was calculated as the percentage ratio of the area under the dissolution curve up to time *t* to that of the area of the rectangle described by 100% dissolution at the same time (Khan 1975) as follows (3):

$$\%DE = \left(\frac{\int_0^t \mathrm{X}\, dt}{y\ 100\ \mathrm{X}\ t}\right) \mathrm{x}\ 100 \quad (3)$$

A well-known method is the trapezoidal method (4). The area under the curve (AUC) (Anderson et al., 1998) is the sum of all the trapezia defined by

$$AUC = \sum_{i=1}^{i=n} \frac{(t_1 - t_{i-1})\ x\ (y_{i-1} + y_i)}{2} \quad (4)$$

where t_i is the i^{th} time point and y_i is the percentage of product dissolved at time t_i.

The mean dissolution time (MDT) was calculated via the following expression (Costa et al., 2003):

$$MDT = \frac{\sum_{i-1}^{n} t_{mid}\ \Delta M}{\sum_{i-1}^{n} \Delta M} \quad (5)$$

where *i* is the dissolution sample number, *n* is the number of dissolution times, t_{mid} is the time at the midpoint between times t_i and t_{i-1}, and ΔM is the amount of MX dissolved (mg) between times t_i and t_{i-1}.

4.2.12 *In vitro* assessment of lung depositon

In vitro aerodynamic assessment was carried out by two methods. The Next Generation Impactor (NGI) was applied in the case of the optimization study, and the Andersen Cascade Impactor (ACI) for stability testing. The impactors are specified in the USP Chapter <601> and Ph. Eur. Chapter 2.9.18 for use in measuring the mass distribution of pharmaceutical aerosols via the aerodynamic diameter.

Next Generation Impactor

The mass of the powder was 3 mg. The products were filled into hard gelatine capsules (size 3). The inhaler device applied was a plastic RS01 (Plastiape, Italy). A Next Generation Impactor (MSP Corp., Minneapolis, MN, USA) fitted with a stainless steel 90 degree induction port was used according to the manufacturer's instructions, which included setting the flow rate at 60 l min^{-1}. During impact, the aerosol was divided into seven size categories according to the aerodynamic diameter of the particles (USP 29). Prior to use, the final stage of the impactor was fitted with a fibreglass filter (nylon, 0.45 µm, Millipore, UK) and seven stages were coated with a 1% w/v Span 85-cyclohexane mixture to control particle rebound.

The NGI was assembled and connected to a vacuum pump (High-capacity Pump Model HCP5, Critical Flow Controller Model TPK, Copley Scientific Ltd., Nottingham, UK). The actual flow rate through the impactor was measured by a mass flow meter (Flow Meter Model DFM 2000, Copley Scientific Ltd., Nottingham, UK) prior to each run, to ensure that the desired flow of 60 l min^{-1} was achieved. The NGI impactor has a range of cut-off diameters as shown in **Table VIII**. The DPI device was connected to the impactor via an airtight rubber adaptor. The pump was started and the initial pressure drop caused by the pump allowed dose release. Each capsule was filled with an amount of product corresponding to ~ 3 mg of powder. Each formulation was actuated into the device through the induction port two times, after which a single experiment was deemed complete. The impactor was dismantled and the inhaler, the mouthpiece, the induction port and each of the seven stages were washed with methanol/buffer (60/40 v/v %) to collect the deposited drug. The micro orifice collector (MOC) was fitted with a filter (nylon, 0.45 µm, Millipore, UK). All samples were quantified by UV/Vis spectrophotometry.

Table VIII: Particle size cut-off point for each stage at a flow rate of 60 l min^{-1} in the NGI.

NGI stage	Cut-off diameter at 60 l min^{-1} (μm)
1	8.06
2	4.46
3	2.82
4	1.66
5	0.94
6	0.55
7	0.34
MOC	0.00

Andersen Cascade Impactor

The aerosol size distribution of different cumulative doses of microcomposites was assessed by using the three cascade impactor methodology with the Andersen Cascade Impactor (Copley Scientific Ltd., Nottingham, UK). At 60 l min^{-1}, the ACI impactor has a range of cut-off diameters as shown in **Table IX**. The flow rate was set to 60 l min^{-1} using a vacuum pump (High-capacity Pump Model HCP5, Critical Flow Controller Model TPK, Copley Scientific Ltd., Nottingham, UK). The actual flow rate through the ACI was measured with a mass flow meter (Flow Meter Model DFM 2000, Copley Scientific Ltd., Nottingham, UK) prior to each run, to ensure that the desired flow of 60 l min^{-1} was achieved.

Table IX: Particle size cut-off point for each stage at a flow rate of 60 l min^{-1} in the ACI.

ACI stage	Cut-off diameter at 60 l min^{-1} (μm)
-1	9.0
0	5.8
1	4.7
2	3.3
3	2.1
4	1.1
5	0.7
6	0.4
Filter	< 0.4

A variety of parameters were employed to characterize the deposition profile of MX: The recovered dose (RD) was the sum of the drug collected from the capsule, the inhaler device, the induction port and all stages of the impactor. The emitted dose (ED) was the amount of drug released from the inhaler device. The fine particle dose (FPD) was defined as the amount of drug deposited on stage 2 and below the impactor. The respirable fraction was expressed in terms of the FPF, deemed to comprise the particles smaller than 5 μm, which was calculated as the ratio of FPD and RD. The total percentage recovery of the drug was assessed as the ratio of the RD to the theoretical dose, the latter being the dose of MX in the capsules. The real dose of MX in one capsule was 1.20 ± 0.38 mg, which was equivalent to the filling weight of co-spray-dried samples (3.29 ± 0.26 mg, depending on the drug content). The MMAD, defined as the median particle diameter of the formulation deposited within the impactor, was determined by interpolation of the percentage undersize versus the logarithmic aerodynamic diameter data between stages 2 and 3. The geometric standard deviation (GSD) was calculated as the square root of the ratio of the particle size at the 84.13^{th} percentile to the 15.87^{th} percentile. Both MMAD and GSD were determined from the linear region of the plot of the cumulative mass distribution as a function of the logarithm of the aerodynamic diameters.

4.2.13 Stability testing

Stability tests were carried out as recommended by the international guidelines specified in ICH (International Conference on Harmonization) Q1A (R2) - Stability Testing of new Drug Substances and Products. Accelerated testing was performed at 40 ± 2 ºC with 75 ± 5% RH. Under both conditions, samples were stored in hard gelatine 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.

4.2.14 Statistical analysis

All measurements were carried out in triplicate and values are reported as means ± S.D. unless otherwise noted. Statistical calculations were performed with the software Statistical for Windows. To identify statistically significant differences, one-way ANOVA with t-test analysis was performed. Probability values of $p < 0.05$ were considered significant.

5. RESULTS

5.1 Preformulation studies of M-based co-spray-dried samples

The wettability and the rate of dissolution of MX are the factors primarily limiting its formulation for pulmonary administration. The main aim of our research was to achieve an adequate crystal habit, good wettability and the rapid release of MX, whereby to achieve better bioavailability in the respiratory system. Another objective was to reduce the amount of the water-soluble carrier M to cover the homogeneously distributed MX.

In particle engineering, co-spray-drying is a one-step process via spray-drying, which can be used as a formulation platform to improve the particle habit of formulations containing MX and excipients together. The main advantage of co-spray-drying is to improve the processability and bioavaibility of MX through the production of spherical, micronized particles coated by additives.

The *particle size analysis* revealed that the crystal sizes of the MX and its products were decreased significantly (**Table X**).

Table X: Particle sizes of samples and MX in products

Sample	Particle size of sample [μm]	SD [±]	Particle size of MX in sample [μm]	SD [±]
MX	85.39	6.63	-	-
MX spd	19.05	2.63	-	-
M	86.74	5.12	-	-
M spd	1.61	0.81	-	-
MX-M	1.37	0.31	4.07	3.50
MX-M-TWEEN	4.11	1.97	1.84	0.34
MX--M-PVP	4.05	1.79	2.93	0.53
MX-M-PVP-TWEEN	5.18	3.95	3.01	0.25

The size of the MX crystals was 85 μm, i.e. nearly the same as for M. The particle size of the MX was first decreased with the UT in an aqueous suspension without additives (24000 rpm for 10 min). After spray-drying of the aqueous suspension, the size of the MX spd was decreased only to 19 μm, because of the poor wettability and aggregation of MX. Spray-drying of the aqueous solution of M resulted in a size of 1.61 μm (M spd). It is interesting to

compare the size of MX-M (1.37 μm) with that of extracted MX (4.07 μm), which demonstrated the aggregation of MX.

The *wettability study* (**Table XI**) indicated that the microcomposites had a more hydrophilic character as compared with MX. Significantly lower contact angles with water were measured for all samples, the decrease ranging from 52º to 22º. The contact angle of MX (75.3º) was relatively high, showing its poor hydrophilicity. Following spray-drying, the MX particle size was decreased significantly, from 85 to 19 μm (**Table X**), but the wettability did not change significantly (72.0º). As concerns the co-spray-dried products, MX had a contact angle of 54.9º because of the wetting character of M. With TWEEN, we observed nearly the same contact angles (19.1º and 20.9º) in both cases and the wetting character could also be perceived. PVP can help stabilize the decreased size and improve the wettability too (26.3º). The polarity of MX spd was increased slightly (20.9%) as compared with the raw MX. Co-spray-drying with M improved the polarity a little (31.4%). With TWEEN and PVP, similar surface free energy and polarity values were manifested as in the case of pure M (~44%).

Table XI: Contact angles, surface free energies and polarities of the materials.

Sample	Θ_{water} [˚]	$\Theta_{diiodomethane}$ [˚]	γ^d [mN m^{-1}]	γ^p [mN m^{-1}]	γ [mN m^{-1}]	Polarity [%]
MX	75.27±5.23	16.23±3.28	44.74	9.25	53.99	17.13
MX spd	72.00±0.00	24.83±2.93	42.21	11.12	53.33	20.85
M	21.34±4.74	17.55±6.38	43.70	33.95	77.65	43.72
M spd	20.13±4.98	24.87±5.13	41.64	35.06	76.70	45.71
MX-M	54.90±6.13	23.90±2.77	42.17	19.32	61.49	31.41
MX-M-TWEEN	19.08±1.44	16.35±0.21	43.97	34.57	78.54	44.01
MX-M-PVP	26.35±4.58	28.00±1.54	40.61	33.22	73.83	44.99
MX-M-PVP-TWEEN	20.93±3.34	18.40±0.89	43.49	34.16	77.65	43.99

The *dissolution* of raw MX at pH 7.4 was prolonged. MX spd exhibited nearly the same profile as that of raw MX, but the reduced size with the increased specific surface yielded a level of 30% in the dissolution. **Fig. 10** presents the dissolution profiles of MX, MX spd and the co-spray-dried systems.

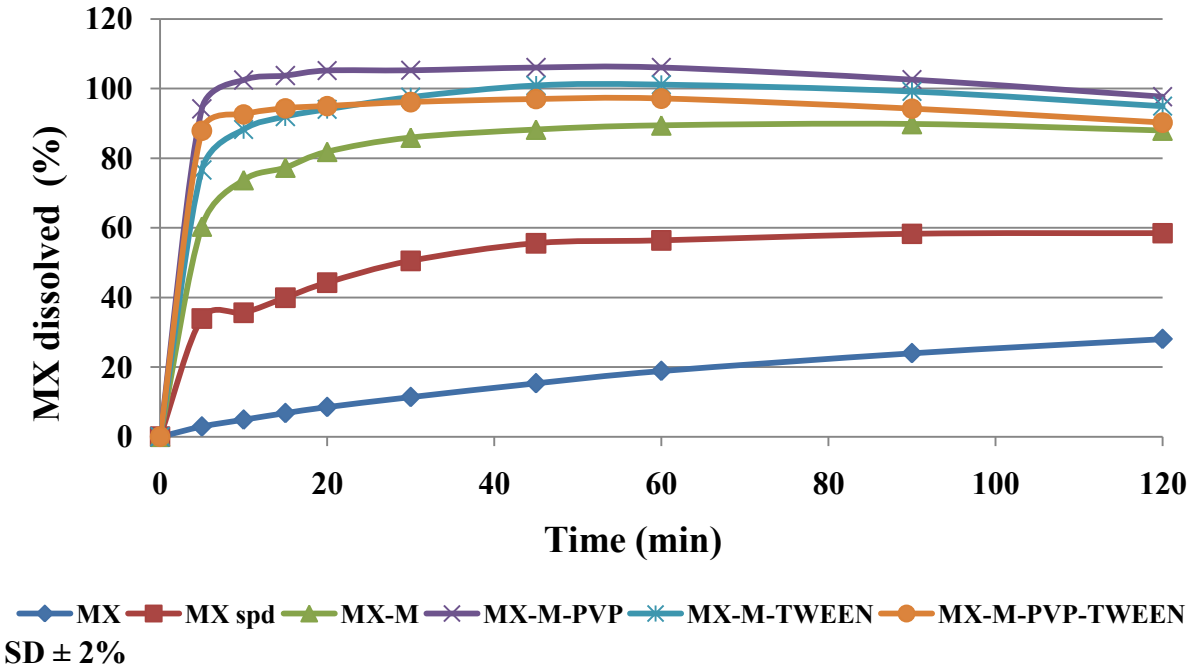


FIGURE 10: Extent of dissolution of MX from co-spray-died samples.

Table XII presents the drug release from the physical mixtures (PM) at 15, 60 and 120 min. Differences in dissolution rate and dissolved concentration of MX was observed between the co-spray-dried samples and the physical mixtures. The products containing the additives gave close to 100% release in the first 5 min. The dissolution in the first 5 min was ~30 times higher than that for MX (**Fig, 10**).

It may be seen that small amounts of additives can play a great role in the dissolution of the drug, and it is therefore important to achieve a correct formulation. PVP itself is not such a good wetting agent as TWEEN. The inhibition of aggregation inhibition by these excipients is necessary as concerns the particle size decrease and protection, which results in enhanced dissolution.

Table XII: *In vitro* drug release of MX from the physical mixtures.

Sample	15 min (%)	60 min (%)	120 min (%)
MX	6.8	18.88	28.04
PM-MX-M	8.13	33.16	55.07
PM-MX-M-TWEEN	46.15	67.85	69.33
PM-MX-M-PVP	27.88	41.38	43.51
PM-MX-M-PVP-TWEEN	52.57	76.37	78.64

5.2 Determination of cytotoxicity of spray-dried MX and microcomposites

Cytotoxicity testing is required for the novel inhalation delivery systems. In the human lung, the submucosal glands are a major source of airway surface liquid, mucins and other immunologically active substances, and Calu-3 cells reflect these properties. The cytotoxicity of MX-containing microcomposites was studied on monolayers of Calu-3 cells, in order to acquire information on the availability in pulmonary formulations. To compare the cytotoxicity with the drug dissolution, the applicable amount of MX was determined for dry powder inhalation. PVP and TWEEN as stabilizing and wetting agents can be safely applied for pulmonary use; toxic effects are not detected in the respiratory tract (Robinson et al., 1990).

To check on the applicability of microcomposites, we investigated the toxicity of samples on Calu-3 lung epithelial cells. Ten different concentrations of products between 0.01 and 50 mg ml^{-1} were applied, i.e. 0.005-25 mg ml^{-1} MX content. **Fig. 11** shows that, up to a concentration of 0.1 mg ml^{-1} all of the products are safely applicable in the lung.

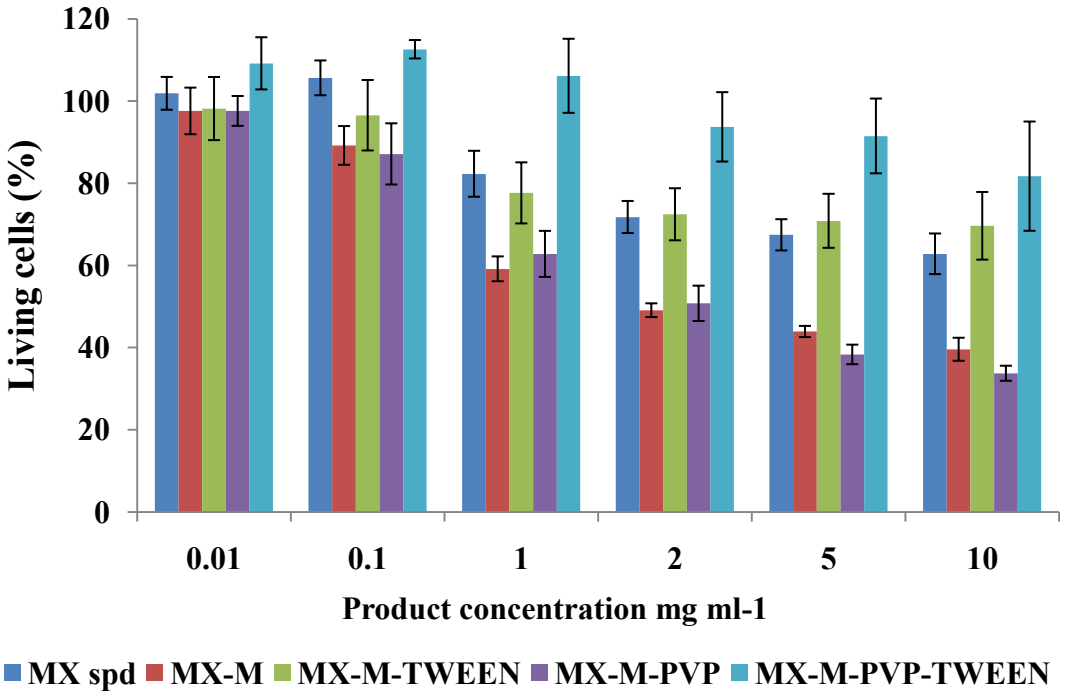


FIGURE 11: Cytotoxicity of MX spd and products containing MX.

Between 1 and 10 mg ml^{-1}, only the MX-M-PVP-TWEEN products resulted in more than 80% cell viability. However, there was a large decrease in the living cell concentration when both TWEEN and PVP were applied between 10 and 25 mg ml^{-1} as compared with the other products. Above 10 mg ml^{-1}, all of the samples were toxic. During the dissolution

testing under alveolar conditions (37 °C, pH 7.4), the 1.67 mg MX content in 100 ml of medium (which is one-tenth of the oral dose) means 0.036 mg ml^{-1} product containing 0.0167 mg ml^{-1} MX. The cytotoxicity tests indicated that, in the case of MX-M-PVP-TWEEN, the dose can be increased up to 10 mg ml^{-1}, including a maximum of 5 mg ml^{-1} MX, and the applicable amount of this drug can therefore be increased 300-fold through use of our formulation.

5.3 Optimization of the product parameters and applied additives for the aerodynamic assessment of MX

The rate of dissolution of MX microcomposites was improved through the use of additives such as PVP and TWEEN. The aerodynamic properties of these samples were characterized: MMAD was < 4.21 ± 0.01. Nevertheless, the respirable fraction was low; the FPFs for the samples were < 30.8 ± 4.44. The aerodynamic assessment of the microcomposites was improved by optimization of the preparation protocol and composition.

The aim of this study was to develop respirable microcomposites of MX and adjuvants (different polymers and an amino acid) for inhalation as drug delivery systems for local lung therapy. MX was transformed into microcomposites, i.e. crystals of drug embedded in M and other adjuvants. We focused on the influence of the concentrations of the polymers on the physicochemical properties of the microparticles. The objective was to optimize the aerodynamic parameters of the particles and to achieve the rapid release of MX. Besides PVP and TWEEN, PVA, as a microfine coating material for MX crystals and LEU were applied during co-spray-drying. Amino acids such as LEU can be co-spray-dried with certain active compounds to modify drug aerolization behavior (Seville et al., 2007, Prota et al., 2011).

The preparation protocol was optimized by cavitation, as the effective particle reducing procedure; to achieve a homogeneous particle size distribution and an enhanced FPF of the formulations (see **Fig. 9**).

The drug content is a limiting factor in the case of spray-drying. The drug contents of the spray-dried powders are shown in **Table XIII**. Values close to 50% were expected. In reality, depending on the additives applied, different values of MX content were observed. It can be seen that without additives (MX-M) the highest loss of MX resulted after spray-drying. This significant loss of MX was ascribed to the poor wettability of the drug during the preparation of the suspension for spray-drying. Separations of the solid phases and MX

adhesion to the glass walls of the spray-dryer were observed. With a surface-active agent in the solution, the real and theoretical MX contents were closer.

Table XIII: Drug contents in the spray-dried powders.

Product	Drug content (% ± SD)
MX-M	27.74 ± 0.06
MX-M-PVP	48.95 ± 0.39
MX-M-PVP-LEU	47.43 ± 0.46
MX-M-TWEEN	47.11 ± 0.57
MX-M-TWEEN-LEU	33.95 ± 1.36
MX-M-PVA	47.69 ± 0.38
MX-M-PVA-LEU	37.02 ± 0.97

The mean size D(0.5) of the raw MX crystals was 30.85 μm, which was nearly the same as for M (**Table XIV**). Microcomposites were obtained by spray-drying. D(0.9) was increased in the case of MX-M due to the aggregation of the particles. The D(0.9) value of the MX-M sample in the suspension was 179.75 μm, while after spray-drying it was 243.68 μm. In the other cases, the additives exerted an aggregation-inhibitory effect during the procedure. Size analysis of the co-spray-dried samples revealed that the particle size was typical of micronized powders (< 10 μm). The distribution became homodisperse when LEU was applied.

Table XIV: Particle size distributions of dried powders.

Product	D(0.1) (μm)	D(0.5) (μm)	D(0.9) (μm)
MX	8.56	30.85	63.71
M	5.32	36.37	65.45
MX-M	1.38	3.09	243.68
MX-M-PVP	1.65	3.29	6.19
MX-M-PVP-LEU	1.53	3.05	5.68
MX-M-TWEEN	2.48	5.09	10.52
MX-M-TWEEN-LEU	2.08	4.16	8.88
MX-M-PVA	1.57	3.11	5.81

The microcomposite formulations after the spray-drying procedures exhibited water contents ranging between 0.23% and 0.64% (**Table XV**). MX-M-TWEEN and MX-M-TWEEN-LEU displayed the highest water contents (0.43% and 0.64%) as TWEEN exerts a hygroscopic effect in the sample during the drying. Lower moisture contents were detected in the polymer-containing formulations, because hygroscopic growth can be prevented by coating the drug particles with polymer layers. Moisture sorption also affected the drug dispersion and de-aggregation behaviour, which in turn impacted on the respiratory drug delivery performance.

Table XV: Residual water contents (%) in the co-spray-dried formulations.

Formulation	Residual water content (%)
MX-M	0.23
MX-M-PVP	0.28
MX-M-PVP-LEU	0.35
MX-M-TWEEN	0.43
MX-M-TWEEN-LEU	0.64
MX-M-PVA	0.29
MX-M-PVA-LEU	0.37

The crystal morphology is a critical parameter for DPI development, because the particle shape affects the aerodynamic behaviour and thus lung deposition. The SEM pictures demonstrated the changes in the habit of the produced microcomposites (**Fig. 12**). The large anisodimensional raw MX crystals (*A*) had a smooth surface with a regular prismatic form, whereas the M crystals had an irregular shape with a rough surface (*B*). The co-spray-dried particles displayed a nearly regular and spheroidal shape. The control product (MX-M) showed the presence of a large number of single spherical particles probably constituted by M alone (*F*). The particles produced with the polymers (PVP and PVA) (*C* and *E*) appeared to be spheroidal without aggregation, and this could be a beneficial feature in the inhalatory application of these powders. The products containing PVA and PVP possessed optimum morphological characters for pulmonary usage (Crowder et al., 2002). The microparticles containing TWEEN had an irregular shape and a stratified surface in consequence of the crystallization-inhibitory effect of the TWEEN concentrated on the surface of the suspension droplets during the spray-drying (*D*). LEU did not modify the morphology of the particles, which were individually separated and displayed a regular size (data not shown). The

spherical and regular forms met the requirements for formulation for DPIs (MX-M, MX-M-PVA, MX-M-PVP and formulations with LEU).

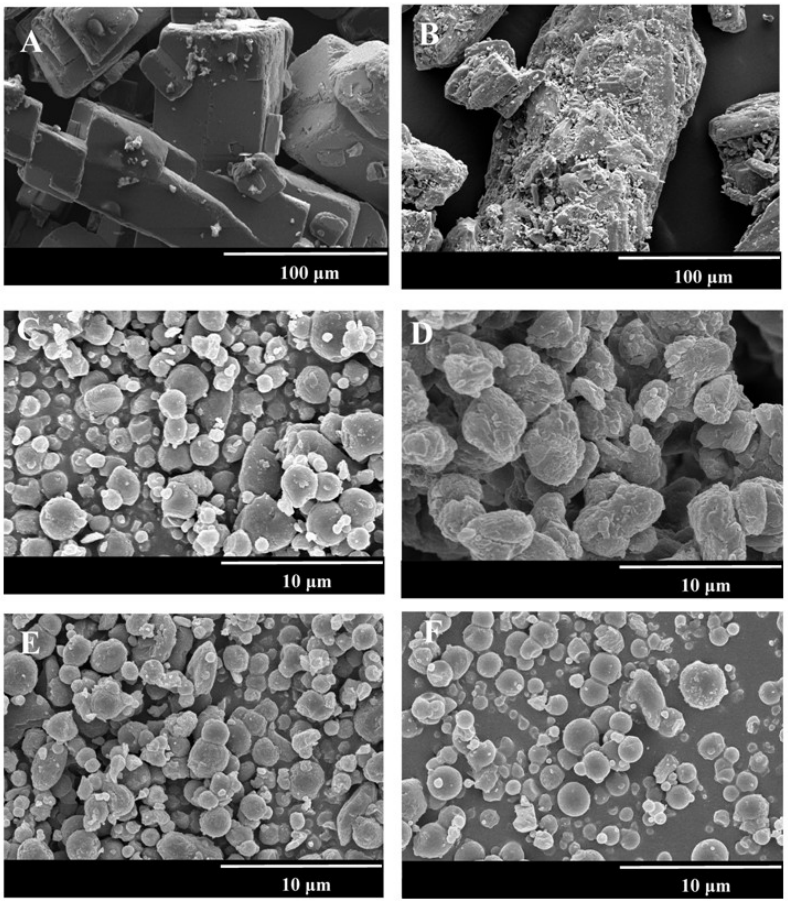


FIGURE 12: SEM images of MX (*A*) and M (*B*) before spray-drying, MX-M-PVP (*C*), MX-M-TWEEN (*D*), MX-M-PVA (*E*), and a control sample of MX after co-micronization with M in a ratio of 1:1 (*F*).

Different polymorphs can be discerned in terms of various physicochemical properties. To reduce the risk of transformation during processing or storage, the polymorphism of the components of the products was controlled. *XRPD investigation* of the products revealed numerous peaks in the spectra (**Fig. 13**). The characteristic peaks of MX were at diffraction angles 2θ of 13.22, 15.06, 26.46 and 26.67, indicating its crystalline structure (Weyna, 2011). The characteristic peaks of β-D-M were clearly observed at diffraction angles 2θ of 10.62, 14.74, 16.9, 21.15, 23.9 and 29.54 (Campbell et al., 2002). The samples containing PVA, PVP and TWEEN induced the α-polymorph form of M. Patterns for the α-polymorph

appeared in these co-spray-dried samples. The peaks for α-M were at: 2*θ of* 9.78, 13.98, 17.44, 20.14, 21.46 and 27.3. Under normal storage conditions, both the α and β forms are considered to be stable (Burger et al., 2000, Hulse et al., 2009)

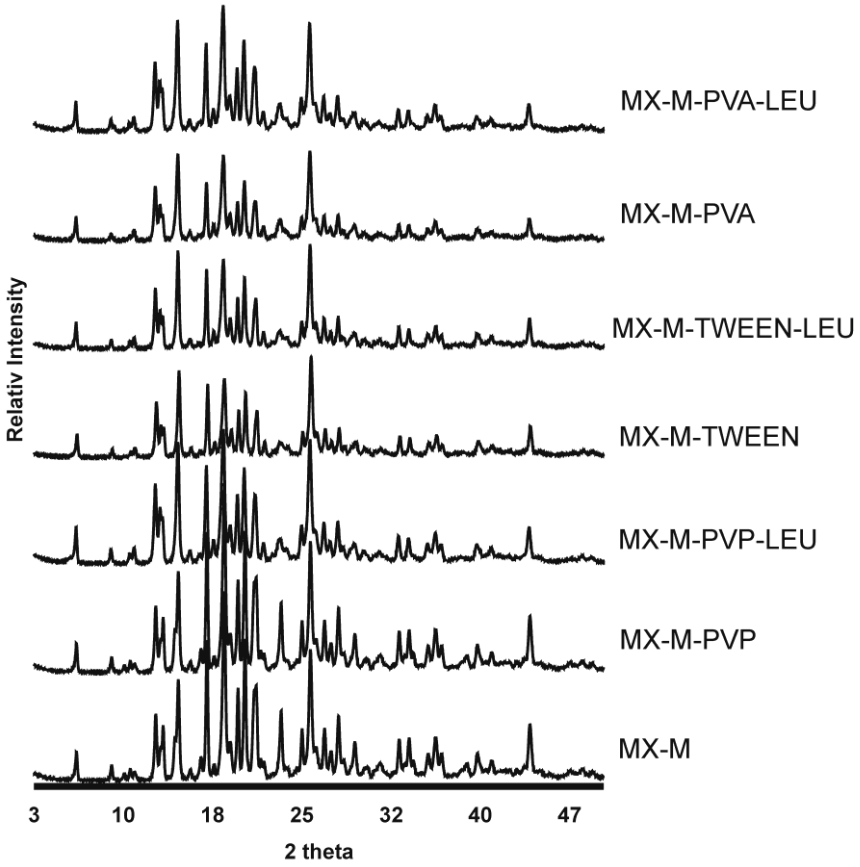


FIGURE 13: XRPD patterns of co-spray-dried products.

The dissolution of raw MX was analysed at pH 7.4 during 45 min. It may be observed in **Table XVI** that the dissolution efficiency at 30 min ($\%DE_{30\ min}$) was enhanced from 8.1 for raw MX to 71.1 for MX-M (as a control) and to 95.3 for the micronized product formulated with TWEEN. Similarly to $\%DE_{30\ min}$, RD at 30 min ($RD_{30\ min}$) was over 80% for the microcomposited products formulated with additives. The difference relative to the control product (MX-M) was significant. $\%DE_{120\ min}$ was > 80% when PVP and PVA were used. $\%DE_{120\ min}$ was highest (97.6 and 90.2, respectively) for MX-M-TWEEN and MX-M-TWEEN-LEU, which also revealed the highest improvement in the rate of MX dissolution.

Table XVI: Percentage dissolution efficiency (%DE), relative dissolution (RD), and mean dissolution time (MDT) of co-spray-dried products in comparison with raw MX.

Products	$\%DE_{10\ min}$	$\%\ DE_{30\ min}$	$\%\ DE_{120\ min}$	$RD_{30\ min}$	MDT
MX	2.9	8.1	17.9	1.0	14.2 ± 1.02
MX-M	59.5	71.1	78.6	8.8	2.7 ± 0.66
MX-M-PVP	74.9	81.7	83.3	10.1	1.3 ± 0.53
MX-M-PVP-LEU	77.0	82.5	83.7	10.2	0.8 ± 0.17
MX-M-TWEEN	88.0	95.3	97.6	11.8	1.1 ± 0.13
MX-M-TWEEN-LEU	77.1	87.8	90.2	10.8	1.8 ± 0.49
MX-M-PVA	71.3	82.3	87.1	10.2	2.1 ± 0.32
MX-M-PVA-LEU	73.2	86.0	91.1	10.6	3.0 ± 0.21

Impactors are used to determine the particle size distribution of aerosols generated from medical inhlers. They directly measure the aerodynamic particle size, which affects how particles move in an airstream. The *in vitro* aerolization properties of the microcomposites were determined according to the Pharmacopoeia test, with the NGI as a multistage impactor model, using the RS01 (Plastiape, Italy) at 60 l min^{-1}. The drug recovery was in the range between 97.2 ± 1.25 and 100.01 ± 2.09%. These values were in the acceptable range (75-125%) for the mass balance according to the quality standard (**Table XVII, XVIII**). The FPFs were between 24.4% and 53.6%, depending on the compositions of the particles. Products formulated with TWEEN displayed low aerolization properties, where the FPF was < 29%, because stratification of TWEEN on the surface caused aggregation. These products were not disaggregated in the air flow, as confirmed by their elevated MMAD values. The FPFs of the control samples (MX-M) were relatively high (43.0 ± 1.1%). The aerolisation performances of the polymer-embedded microcomposites were compared with those of the control. PVP and PVA, as polymer agents, exhibited an aggregation inhibitory effect and ensured the dispersion of individual particles. Such particles underwent disaggregation in the air flow of the device and their FPFs were improved. The highest FPF was that of MX-M-PVA-LEU (57.5 ± 1.0%). The MMAD values of the polymer-embedded systems were between 3.04 ± 0.17 μm and 3.90 ± 0.10 μm.

Fig. 14 and **15** show the amounts of drug deposited on the throat, on stages 1–7 and on the filter, expressed as percentages of the total amount of powder recovered. PVP or PVA formulated with LEU reduced the impact of MX in the throat (< 20%) and increased its deposition in the lowest stages (**Fig. 15**), respectively. With the exception of the samples

containing TWEEN, all of the products exhibited favourable aerolisation characteristics, these powder particles impacting on stages 2-5. The aerodynamic properties of MX-M-PVP and MX-M-PVA were further improved ($p < 0.05$) when LEU was introduced into the formulation. The FPFs were increased, in both cases to more than 53%. The use of LEU as excipient proved effective in reducing the cohesion between the particles and improving the powder dispersion delivered from the DPI.

Table XVII: Loaded amount and delivery from RS01 (n = 3).

Product	Loaded powder (mg)	Emitted powder (mg)	Emitted dose (mg)
MX-M-PVP	3.03 ± 0.18	3.03 ± 0.18	1.48 ± 0.09
MX-M-PVP-LEU	3.25 ± 0.07	3.25 ± 0.07	1.54 ± 0.03
MX-M-TWEEN	3.21 ± 0.23	3.21± 0.23	1.51 ± 0.1
MX-M-TWEEN-LEU	3.55 ± 0.21	3.5 5± 0.42	1.21 ± 0.07
MX-M-PVA	3.31 ± 0.06	3.31 ± 0.06	1.58 ± 0.03
MX-M-PVA-LEU	3.12 ± 0.45	3.12 ± 0.45	1.15 ± 0.16
MX-M	3.40 ± 0.07	3.40 ± 0.14	0.94 ± 0.02

Table XVIII: Deposition of co-spray-dried microcomposites in the ACI at 60 l min^{-1} via RS01 (n = 3).

Product	FPD (mg)	FPF (%)	MMAD (μm)	GSD
MX-M-PVP	0.60 ± 0.07	46.35 ± 4.2	3.90 ± 0.10	1.72 ± 0.30
MX-M-PVP-LEU	0.67 ± 0.01	53.05 ± 1.13	3.52 ± 0.13	2.02 ± 0.02
MX-M-TWEEN	0.24 ± 0.02	24.44 ± 3.81	5.83 ± 0.05	1.90 ± 0.13
MX-M-TWEEN-LEU	0.29 ± 0.05	28.08 ± 1.66	6.18 ± 0.25	2.46 ± 0.06
MX-M-PVA	0.64 ± 0.06	53.53 ± 2.02	3.39 ± 0.11	2.01 ± 0.01
MX-M-PVA-LEU	0.54 ± 0.03	57.5 ± 1.0	3.04 ± 0.17	2.23 ± 0.04
MX-M	0.31 ± 0.1	43.01 ± 1.06	3.74 ± 0.01	2.27 ± 0.01

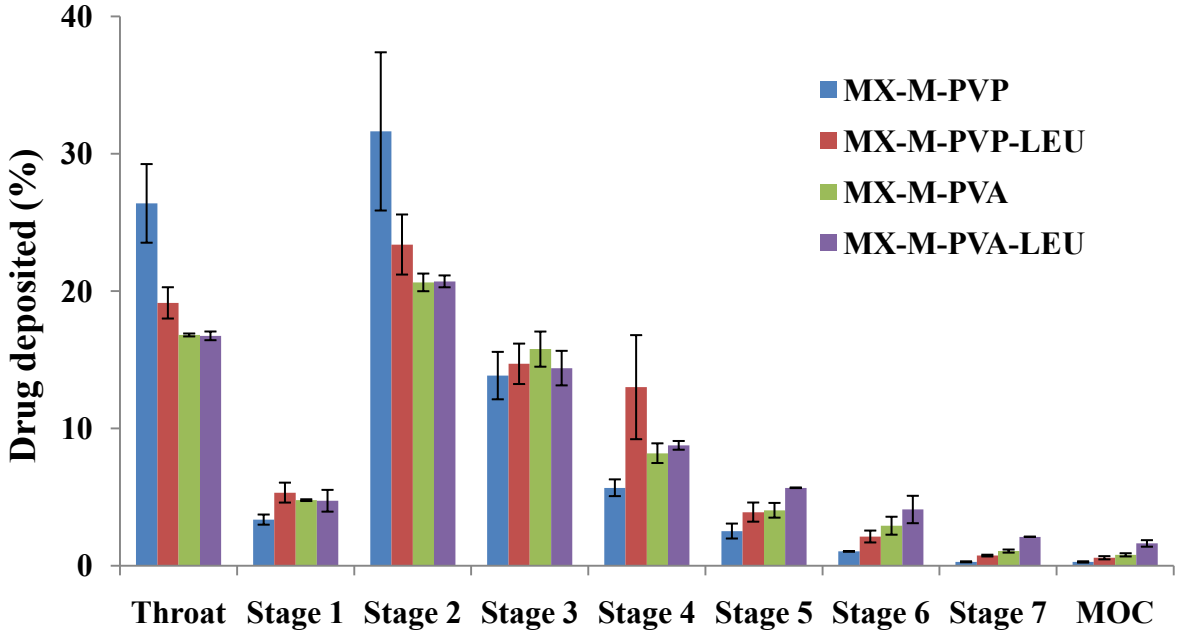


FIGURE 14: Drug deposition profiles of co-spray-dried microcomposites in the NGI at 60 l min^{-1} via the RS01 (n = 3).

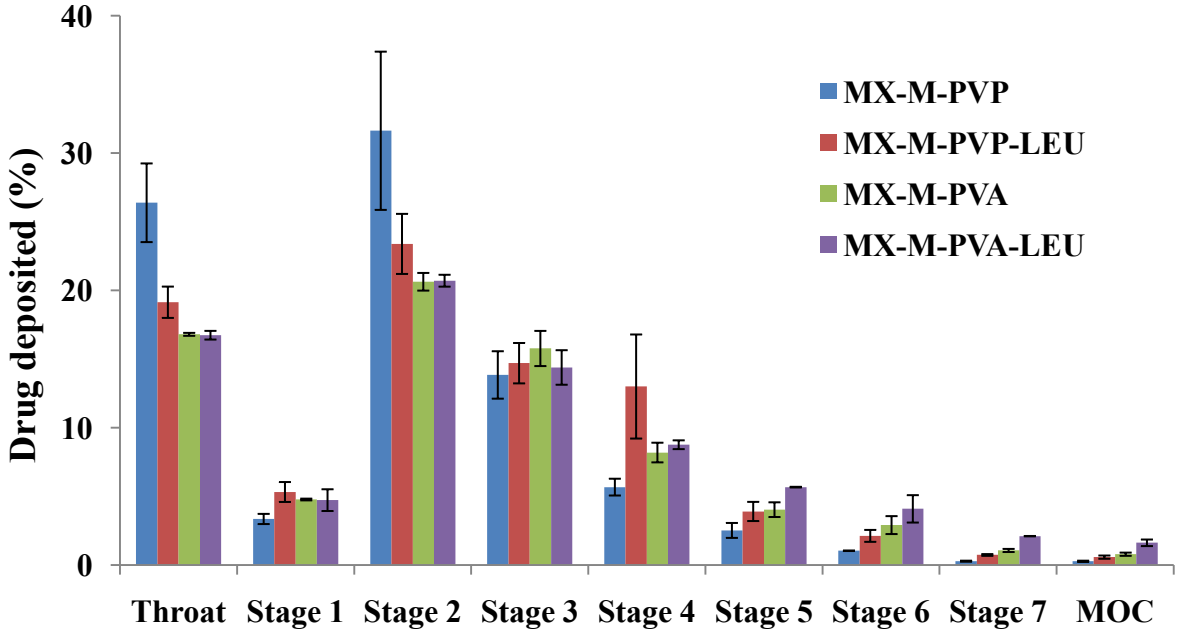


FIGURE 15: Drug deposition profiles of co-spray-dried microcomposites in the NGI at 60 l min^{-1} via the RS01 (n = 3).

5.4 Physicochemical stability testing and influence of humidity and temperature on aerodynamic properties

The aim of the present work was the accelerated stability testing of the M-based co-spray-dried products containing MX as DPI forms. The samples that were investigated were chosen in a optimization study. We examined the influence of the RH and temperature on the physicochemical properties and aerolization parameters of the co-spray-dried microcomposites during storage. Our overall aim, based on the results of the stability tests, was to suggest an MX-containing composition as DPI form for further investigation (in vivo test).

The FDA and the European Inhalanda Group have published their agreements on the tests required for the approval of new DPIs. The FDA requires the stability testing of DPI powders, with determination of the appropriate storage conditions and the effects of storage on the particle size distribution, including the effects of moisture (FDA 1998, Inhalanda 1998, Byron 1994, Ashurst et al., 2000).

The storage conditions during stability testing are determined on the basis of the ICH harmonized Guideline of Stability Testing of New Drug Substances and Products Q1A (R2). The ICH Guideline specifies the following storage conditions for accelerated tests: 40 ± 2 ºC with 75 ± 5% RH. Samples are stored in hard gelatine capsules in open containers; the duration of storage is 6 months (Braun et al., 1996, Hindle et al., 1996, Young et al., 2004, Lida et al., 2004, Young et al., 2003, Zeng et al., 2007, Das et al., 2009).

The long-term storage conditions: 25 ± 2 ºC with 60 ± 5% RH, for a minimum period of 12 months. The results of long-term stability testing have not been described in recent studies. We discussed the accelerated stability testing of the M-based co-spray-dried products containing MX as DPI forms. We examined the influence of the RH and temperature on the physicochemical properties and aerolisation parameters of the co-spray-dried microcomposites during storage. The effects of PVA and PVP were additionally investigated in terms of particle size, shape, physicochemical stability and aerolisation of the DPI form by using the ACI.

The most important parameter of the stored samples is the residual water content. The microcomposite formulations before storage exhibited water contents ranging between 0.35% and 0.37% (**Table XIX**). For MX-M-PVP-LEU, the change in water content was higher than that for MX-M-PVA-LEU. MX-M-PVA-LEU is more resistant to the RH and higher temperature because of the low solubility of PVA in the hydrophilic medium. In contrast, as a

water-soluble agent, PVP can absorb the water on its surface. Moisture sorption also affected the drug dispersion and de-aggregation behaviour, which in turn impacted on the respiratory drug delivery performance.

Table XIX: Residual water content in co-spray-dried samples.

Formulation	Residual water content (%) Before storage	Residual water content (%) After storage
MX-M-PVP-LEU	0.35	0.64
MX-M-PVA-LEU	0.37	0.45

Since the two samples were prepared under similar spray-drying conditions, the final particle size distributions of the samples were similar. Size analysis of the co-spray-dried samples revealed that the particle size was typical of micronized powders (< 10 μm) (**Table XX**). The median volume diameter of MX-M-PVP-LEU before storage was 3.05 μm, and that after storage was 3.58 μm. The D(0.5) of MX-M-PVA-LEU before storage was 3.11 μm, and that after storage was 3.38 μm (n = 3).

The particle size distribution indicated monodisperse particles (Span ≤ 1.36). The Span values were nearly the same and exhibited a relatively uniform size distribution before and after storage; the change in distribution was 0.16 ± 0.9 for MX-M-PVP-LEU, and 0.06 ± 0.13 for MX-M-PVA-LEU.

Table XX: Particle size of co-spray-dried samples before and after storage.

Sample	D(0.1) μm	D(0.5) μm	D(0.9) μm	Span
MX-M-PVP-LEU Before storage	1.53	3.05	5.68	1.36 ± 0.3
MX-M-PVP-LEU After storage	1.75	3.58	6.03	1.20 ± 0.6
MX-M-PVA-LEU Before storage	1.64	3.11	5.81	1.34 ± 0.4
MX-M-PVA-LEU After storage	1.79	3.37	6.11	1.28 ± 0.9

A representative SEM image of the M-based particles is shown in **Fig. 16**. The morphology of the co-spray-dried samples was determined by the M, which recrystallized on the surface of the MX microcrystals during the co-spray-drying process. In general, spray-

dried M has a spherical morphology with a clearly crystalline structure, typical of this carrier. The polymers (PVA and PVP) modified the surface of the MX co-sprayed with M and exerted an aggregation-inhibitory effect, thereby furnishing individual particles.

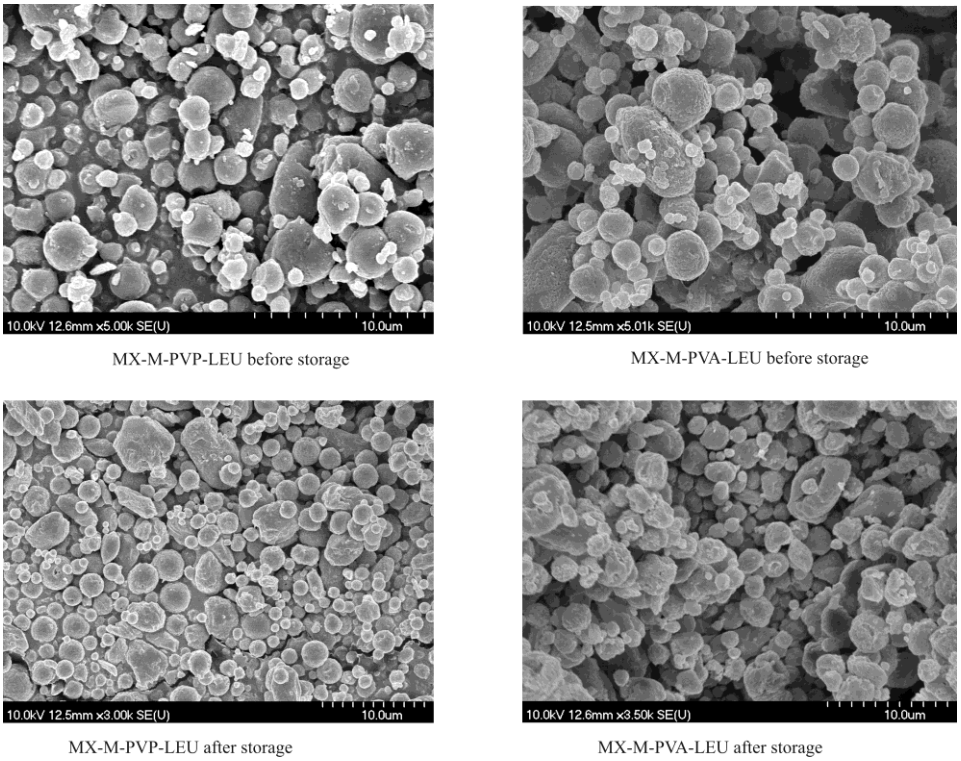


FIGURE 16: SEM of microcomposites before and after storage.

To investigate the interactions between MX and the polymers (PVP and PVA), we compared the FT-IR spectra of the formulations before and after storage (**Fig. 17** and **18**). The FT-IR spectrum of MX exhibited distinct peaks at 3291 cm^{-1}, 1620 cm^{-1} (N-H) and 1580 cm^{-1} (C-O). In the prepared samples, MX had a crystalline form, i.e. its micronization and co-spray-drying process did not result in an amorphous product. The intensity increase in the interval 3500-3100 cm^{-1} was induced by the co-spray-drying procedures, which resulted in the increases in the associated O-H bonds and H-bonding interactions in the solid state between MX and the different polymers. The spectra revealed no difference in the positions of the absorption bands, especially with respect to OH, =O and NH, thereby providing evidence of the absence of H-bonding interactions in the solid state between the polymer and MX. The interactions between the surface groups of PVP and PVA and the OH groups of MX on the surface do involve H-bonding (Jafar et al., 2010). In MX-M-PVP-LEU, the interactions are reduced during storage, as indicated by the decreases in the associated O-H bonds and H-

bonding in the interval 3500-3100 cm^{-1}. The H-bonding did not change during storage in the case of MX-M-PVA-LEU (Forster et al., 2001).

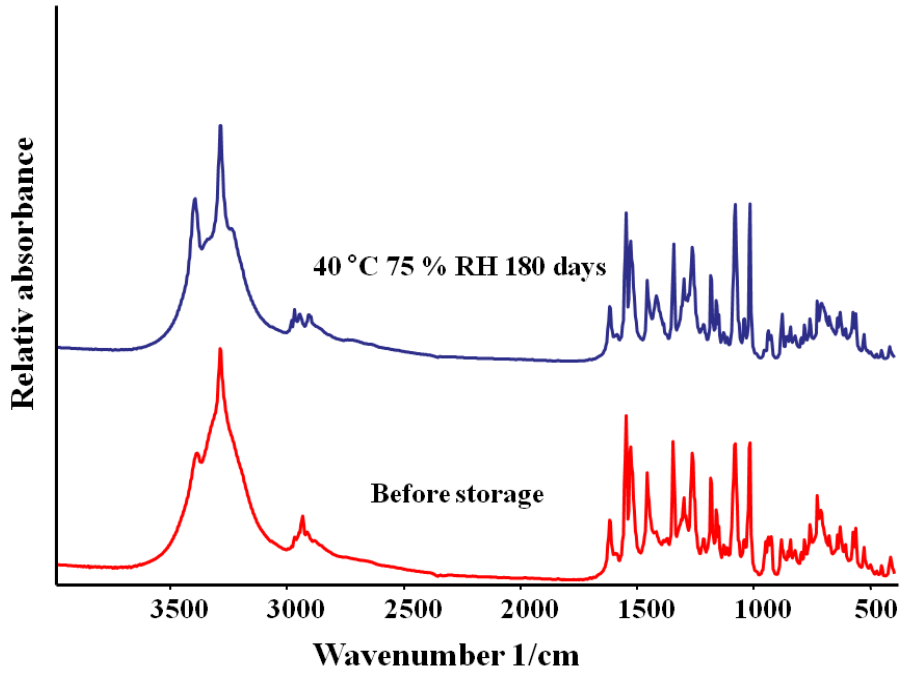


FIGURE 17: FT-IR analysis of MX-M-PVP-LEU before and after storage. The characteristic range is 3500-3100 cm^{-1}.

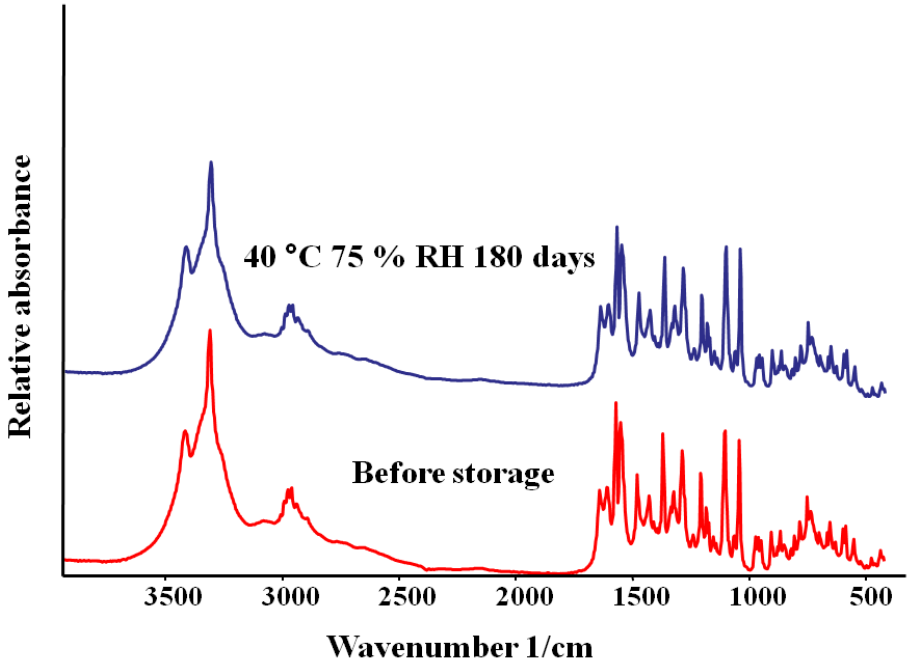


FIGURE 18: FT-IR analysis of MX-M-PVA-LEU before and after storage. Associated O-H bonds and H-bonding in the interval is 3500-3100 cm^{-1}.

The *in vitro* aerolisation properties of the microcomposites were determined as specified in the Pharmacopoeia test. **Table XXI** shows the specific aerodynamic properties.

Table XXI: Deposition of co-spray-dried microcomposites in the cascade impactor at 60 l min^{-1} via RS01 (n = 3).

Product	FPD (mg)	FPF (%)	MMAD (μm)
MX-M-PVP-LEU before storage	0.60 ± 0.07	53.05 ± 1.13	3.52 ± 0.13
MX-M-PVP-LEU after storage	0.24 ± 0.02	41.16 ± 2.81	3.91 ± 0.45
MX-M-PVA-LEU before storage	0.64 ± 0.05	57.50 ± 1.0	3.04 ± 0.17
MX-M-PVA-LEU after storage	0.54 ± 0.03	52.5 ± 2.2	3.39 ± 0.11

Before storage, the FPF of MX-M-PVP-LEU was 53.05 ± 1.13% and the MMAD was 3.52 ± 0.13 μm. After storage, the MMAD of the PVP-embedded formulations was 3.91 ± 0.45 μm. After storage, the FPFs of the samples were reduced because of the fine particle aggregation. This is connected with the increased capillary interaction and/or moisture adsorption of PVP at higher RH (75%). The FPF of the PVA-containing samples (MX-M-PVA-LEU) was relatively high before storage (57.50 ± 1.0%) and after storage at 40 ºC and 75% RH was 52.5 ± 2.2%. The MMAD and FPF of the PVA embedded formulations did not change significantly during storage. PVP and PVA, as polymer agents, exhibited an aggregation-inhibitory effect and ensured the distribution of the individual particles. Such particles underwent disaggregation in the air flow of the device and their FPFs were improved. **Fig. 19** and **20** show the amounts of drug deposited on the throat, on stages 0-6 and on the filter, expressed as percentages of the total amount of powder recovered. During stability testing, it can be concluded that PVP or PVA formulated with LEU increased the impact of MX in the device and throat and reduced its deposition in the lowest stages under both conditions. MX-M-PVA-LEU exhibited favourable aerolisation characteristics, these powder particles impacting at a higher rate on stages 2-5, and the changes in deposition in the throat and the device after storage were not pronounced.

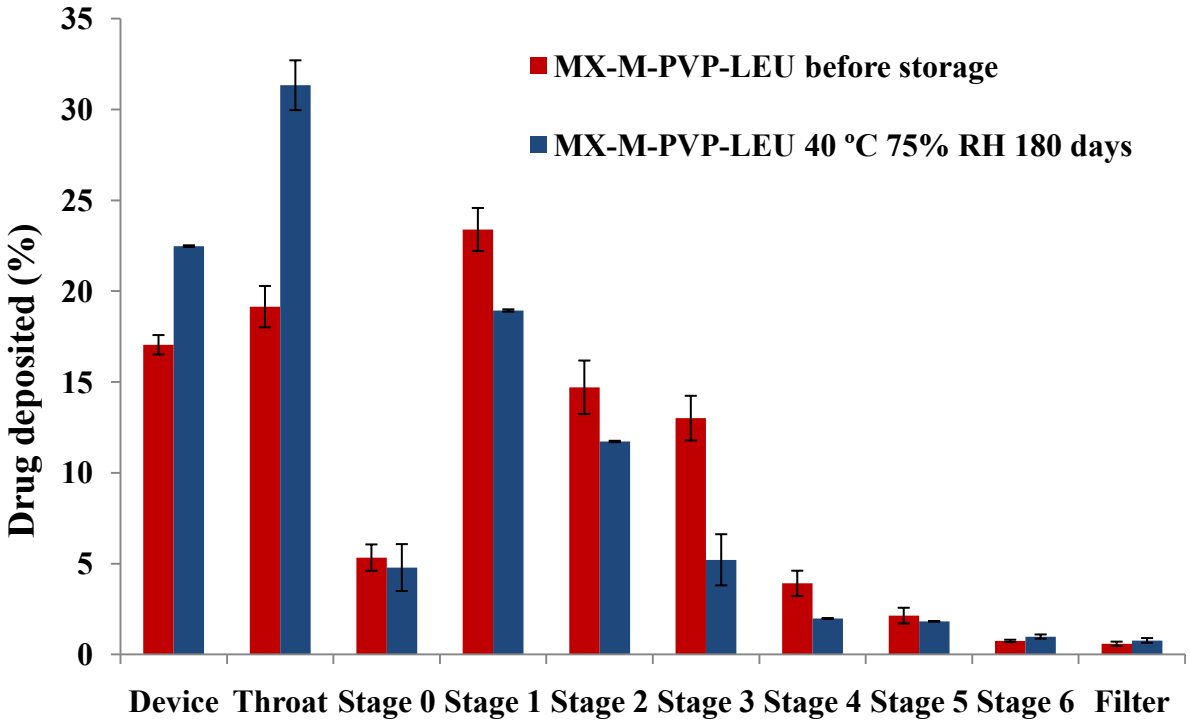


FIGURE 19: Drug deposition profiles of PVP-containing co-spray-dried microcomposites in the ACI at 60 l min^{-1} via the RS01 (n = 3).

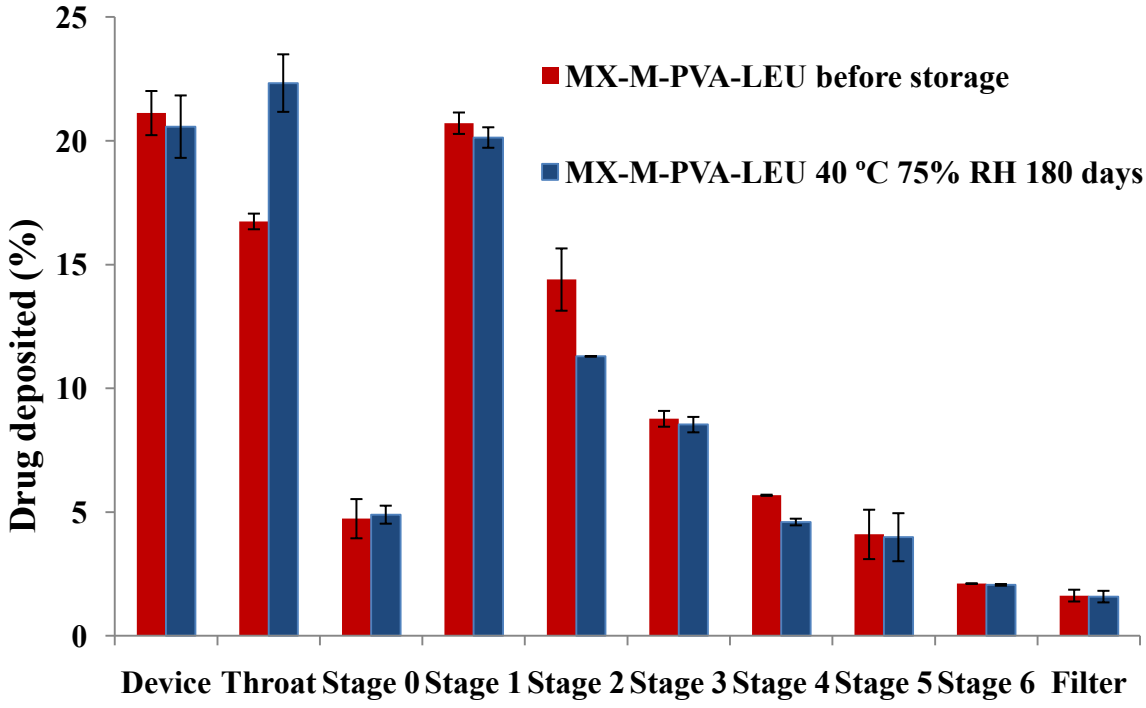


FIGURE 20: Drug deposition profiles of PVA containing co-spray-dried microcomposites in the ACI at 60 l min^{-1} via the RS01 (n = 3)

6. SUMMARY

The primary aim of this study was to carry out research relating to the development of DPI products. We studied the key features of powders for drugs intended for use in pulmonary delivery. Another objective was to develop a carrier-based, crystalline co-spray-dried DPI product containing low-soluble MX. Various approaches to carrier-based formulations were investigated in order to enhance the delivery of MX.

i. In order to produce particles in an adequate size range for pulmonary delivery, the co-spray-drying process was chosen. The particle size and morphology of the MX and its composites were investigated via the additives and the process parameters. Instead of the classical approach based on micronization to prepare drug particles (e.g. grinding, crystallization, etc.), followed by blending with a carrier, co-spray-drying of the MX/M/additives was performed in an one-step process. The dissolved excipients were covered by their adsorption and recrystallization the MX particles to produce solid systems with altered particle size, particle size distribution, morphology and rate of dissolution of the drug. Our results point to an alternative methodology with which to achieve the rapid release of poorly-soluble drugs.

ii. Preliminary cell toxicity studies have shown that the use of additives at the given concentrations has no effect on the viability of Calu-3 cells over a 24 h period. The minimum level of cell viability was higher and non-significantly different from that exhibited with MX alone (~ 70%). The cytotoxicity tests indicated that, in the case of MX-M-PVP-TWEEN, the dose can be increased up to 10 mg ml^{-1}, including a maximum of 5 mg ml^{-1} MX, and the applicable amount of this drug can therefore be increased 300-fold through use of our formulation. It can be concluded that this study has furnished useful information concerning the development of a DPI system containing a MX carrier and additives. Naturally, further *in vivo* and dosage form investigations must be carried out.

iii. The product parameters were optimized with PVA, PVP, TWEEN and LEU as additives in order to improve the drug deposition in the lung. The physicochemical properties and aerolisation efficiency of co-spray-dried M-based formulations including MX were tested as DPI systems for pulmonary drug delivery. We found that the polymers (PVA and PVP) modified the surface of the MX co-sprayed with M and exerted a satisfactory aggregation-inhibitory effect, thereby furnishing individual particles. The presence of LEU further

improved the respirable fraction of MX, and resulted in optimum deposition in the *in vitro* assessment. The mean particle size in the M-based systems was in the required range (3-5 μm). The polymer-embedded MX-M particles were spherical and the MX in the microcomposites had a crystalline structure. This means that the MX was not modified during the wet grinding and co-spray-drying procedure. About 90% of the MX dissolved from the co-spray-dried samples in the first 5 min. The products formulated with TWEEN displayed low aerolisation properties (FPF < 30%) because the stratification of TWEEN on the surface caused aggregation of the particles. The FPFs of the microparticles containing the polymers were increased, in some cases to more than 53%. Overall, this study has demonstrated that the addition of LEU in the MX-M co-spray-dried systems (MX-M-PVA-LEU and MX-M-PVP-LEU) produced co-spray-dried inhalation powders containing composite particles applicable for bronchial deposition.

iv. The accelerated stability testing of M-based co-spray-dried MX-containing DPI systems was performed in order to determine the influence of the RH and temperature on the physicochemical properties and aerolisation parameters. PVP has slightly hygroscopic properties at higher RH; under accelerated conditions (75%), the residual water content of the PVP-containing microcomposites ranged from 0.35% to 0.64%. The PVA-containing M-based sample was more stable against the RH and temperature; the residual water content ranged from 0.37% to 0.45%. The particle sizes of the samples satisfied the pharmacopoeial requirements; no significant increase in size was detected during storage. The polymer-embedded MX-M particles were spherical and the stored products maintained their forms; no changes in surface or aggregation were detected. MX and M in the microcomposites were in crystalline form; the polymer induced the α-polymorph form of M. The FT-IR curves revealed only weak H-bonding between PVP and MX during accelerated storage. The FPF decreased from 53.05 ± 1.13% to 41.16 ± 2.81% during stability testing. The number of H-bonds between PVA and MX did not decrease significantly, FPF at 40 ºC and 75% RH for 6 months was 52.5 ± 2.2%, and MMAD was 3.39 μm. Overall, this study has demonstrated that the aerolisation performance is dependent on the properties of the carrier, the polymer and the dispersity agent (LEU). MX-M-PVA-LEU was stable in the accelerated stability studies; only minor changes were observed in the physicochemical properties and aerodynamic performance.

7. PRACTICAL ASPECTS

This study has demonstrated the ability of micronization of API and spray-drying techniques to produce microcomposites containing crystalline MX and with particle dimensions suitable for pulmonary administration (< 5 μm). The pulmonary application of MX is a novelty for local anti-inflammatory treatment; at present, MX-containing DPI products are not marketed for pulmonary therapy.

- Co-spray-drying of MX from an aqueous microsuspension as innovative technology was used to prepare the novelty-type of microcomposites. The developed technology allows the formulation of a low water-soluble API from a microcrystalline suspension. The method is organic solvent-free and fast. The final crystalline microcomposites is prepared in an one-step process, which additionally ensures the respirable particle size range (3-5 μm), the spherical form and crystallinity property of the product.
- The formulated microcomposite as innovative product reflects is new tendency in pulmonary drug delivery. The hydrophilic carrier (M) and additives (PVP, PVA, LEU) are located on the surface of the API with a hydrophobic character therefore the microcomposites result in the rapid drug release. Crystalline M may also exert a stabilizing effect on the API and it is an osmotic agent; M is also used to enhance clearance the mucus in cases of bronchiectasis and cystic fibrosis. M was primarily chosen as a carrier in this study because its particle size can be readily controlled by spray-drying and the spray-dried powder is crystalline and physically stable. The sample containing PVA proved to be stable in accelerated stability studies, and is an innovative product which may be considered suitable for scaled-up processes and pulmonary application.

The formulated microcomposites illustrate a novel possibility in anti-inflammatory treatment and for the mono- and combination therapy of cancer, pulmonary fibrosis and pain. The innovative technology and product appear to be of great potential in pulmonary, drug delivery systems.

REFERENCES

Adi H, Young PM, Chan HK, Agus H, Traini D. Co-spray-dried mannitol–ciprofloxacin dry powder inhaler formulation for cystic fibrosis and chronic obstructive pulmonary disease Eur J Pharm Sci 2010; 40: 239–247.

Ambrus R, Kocbek P, Kristl J, Sibanc R, Rajkó R, Szabó-Révész P. Investigation of preparation parameters to improve the dissolution of poorly water-soluble meloxicam. Int J Pharm 2009; 381:153-159.

Amidi M, Pellikaan HC, De Boer A, Crommelin DJA, Hennink WE, Jiskoot W. Preparation and physicochemical characterization of supercritically dried insulin-loaded microparticles for pulmonary delivery. Eur J Pharm Biopharm 2008; 68: 191–200.

Anderson SD, Brannan J, Spring J, Spalding N, Rodwell LT. Chan HK, Gonda I, Walsh A, Clark AR. A new method for bronchialprovocation testing in asthmatic subjects using a dry powder of mannitol. Am J Respir Crit Care Med 1997; 156: 758–765.

Arafa HMM, Abdel-Wahab MH, El-Shafeey MF, Badary OA, Hamada FMA. Anti-fibrotic effect of meloxicam in a murine lung fibrosis model. Eur J Pharmacol 2007; 564: 181–189.

Ashurst I, Malton A, Prime D, Sumby B. Latest advances in the development of dry powder inhalers. Pharm Sci Techno To 2000; 3: 246-256.

Aswania O, Chrystyn H. Relative lung and systemic bioavailability of sodium cromoglycate inhaled products using urinary drug excretion post inhalation. Biopharm Drug Dispos 2002; 23: 159–163.

Basavoju S, Bostro D, Sitaram PV. Indomethacin–Saccharin Cocrystal: Design, Synthesis and Preliminary Pharmaceutical Characterization. Pharmaceut Res 2008; 25:530-541.

Bashiri-Shahroodi A, Nassab PR, Szabó-Révész P, Rajkó R. Preparation of solid dispersion by dropping method to improve dissolution rate of meloxicam as poorly water-soluble drug. Drug Dev Ind Pharm 2008; 34:781-788.

Bavbek S, Dursun AB, Dursun E, Erylmiaz A, Misirligil Z. Safety of meloxicam in aspirin-hypersensitive patients with asthma and/or nasal polyps. Int Arch Allergy Immunol 2007; 142: 64–69.

Becker AB, Simons FE. Comparison of formoterol, a new long-acting beta-agonist, with salbutamol and placebo in children with astma. Journal Allergy Clin Immun. 1989; 84: 185.

Bednarek D, Szuster-Ciesielska A, Zdzisinska B, Kondracki M, Paduch B, Kandefer-Szerszen M. The effect of steroidal and non-steroidal anti-inflammatory drugs on the cellular immunity of calves with experimentally induced local lung inflammation. Vet Immunol Immunopathol 1999; 71: 17–28.

Bennett WD, Scheuch G, Zeman KL, Brown JS, Kim C, Heyder J, Stahlhofen W. Regional deposition and retention of particles in shallow inhaled boli: effect of lung volume. 1999; 86: 168-173.

Bianco S. Anti-reactive anti-asthmatic activity of non-steroidal anti-inflammatory drugs by inhalation. United States Patent 2000; 6051566.

Blagden N, de Matas M, Gavan PT, York P. Crystal engineering of active pharmaceutical ingredients to improve solubility and dissolution rates. Adv Drug Deliver Rev 2007; 59:617–630.

Brand P, Friemel I, Meyer T, Schulz H, Heyder J, Haussinger K. Total deposition of Therapeutic Particles During Spontaneous and Controlled Inhalations. J Pharm Sci 2000; 89: 724-731.

Brannan J, Anderson SD, Perry CP, Freed-Martens R, Lassig AR, Charlton B. The safety and efficacy of inhaled dry powder mannitol as a bronchial provacation test for airway hyperresponsiveness: a phase 3 comparision study with hypertonic (4.5%) saline. Respir Res 2005; 6: 144.

Braun M, Oschmann R, Schmidt P. Influence of excipients and storage humidity on the deposition of disodium cromoglycate (DSCG) in the twin impinger. Int J Pharm 1996; 135: 53–62.

Braun DE, Maas SE, Zencirci N, Langes C, Urbanetz NA, Griesser UJ. Simultaneous quantitative analysis of ternary mixtures of d-mannitol polymorphs by FT-Raman spectroscopy and multivariate calibration models. Int J Pharm 2009 385: 29–36.

Buttini F, Soltani A, Colombo P, Mariotti C, Jones SA. Multilayer PVA adsorption onto Hydrophobic drug substrates to engineer drug-rich microparticles. Eur J Pharm Sci 2008; 33: 20-8.

Brewster ME, Loftsson T. Cyclodextrins as pharmaceutical solubilizers. Adv Drud Deliver Rev 2007; 59:645-666.

Brittain HG. Effect of mechanical processing on phase composition. J Pharm Sci 2002; 91:1573-80.

Buckton G. Characterization of small changes in the physical properties of powders of significance for dry powder inhaler formulations. Adv Drug Deliv Rev 1997; 26: 17–27.

Burger A, Henck JO, Hetz S, Rollinger JM, Weissnicht AA, Stöttner H. Energy/temperature diagram and compression behaviour of the polymorphs of mannitol. J Pharm Sci 2000; 89: 457-468.

Byron PR, Patton JS. Drug-delivery via the respiratory-tract. J Aerosol Med- Deposition Clearance and Effects in the Lung. 1994; 7: 49-75.

Byron P. United States Pharmacopeia recommendations for the testing of inhalers. J Aerosol Med 1998; 11: S11–S12.

Campbell RSN, Williams AC, Griemsey M, Booth SW. Quantitative analysis of mannitol polymorphs. X-ray powder diffractometry-exploring preferred orientation effects. J Pharm Biomed Anal 2002; 28: 1149–1159.

Carvalho TC, Peters JI, William RO. Influence of particle size on regional lung deposition – What evidence is there? Int J Pharm 2011; 406: 1–10.

Charokopos N, Apostolopoulos N, Kalapodi M, Leotsinidis M, Karamanos N, Mouzaki A. Bronchial asthma, chronic obstructive pulmonary disease and NF-kappaβ. Curr Med Chem 2009; 16: 867-83.

Chew NYK, Shekunov BY, Tong HHY, Chow AHL, Savage C, Wu J, Chan HK.. Effect of amino acids on the dispersion of disodium cromoglycate powders. J Pharm Sci 2005; 94: 2289–2300.

Chew NYK, Chan HK. Influence of particle size, air flow, and inhaler device on the dispersion of mannitol powders as aerosols. Pharm Res 1999; 16: 1098–1103.

Chiara Parlati. Respirable microparticles of aminoglycoside antibiotics for pulmonary. PhD thesis. Department of pharmaceutical technology, Parma. 2008.

Chow AHL, Tong HHY, Chattopadhyay P, Shekunov BY. Particle engineering for pulmonary drug delivery. Pharm Res 2007; 24: 411-437.

Chrystyn H. The DiskusTM: a review of its position among dry powder inhaler devices. Int J Clin Pract 2007; 61: 1022-1036.

Clarke SW, Newman SP. Therapeutic aerosol 2 - Drugs available by inhaled route. Torax 1984; 39:1-7.

Clarke SW. Medical aerosol inhalers: past present and future. Aerosola and the Lung:Clinical and experimental aspects. 1984; pp: 1-18. London.

Costa FO, Sousa JJS, Pais AACC, Formosinho SJ. Comparison of dissolution profiles of Ibuprofen pellets. J Control Release 2003; 89: 199-212.

Courrier H, Butz N, Vandamme T. Pulmonary drug delivery systems: recent developments and prospects. Critical Reviews in Therapeutic Drug Carrier Systems 2002; 19: 425-498.

Crowder TM, Rosati JA, Schroeter JD, Hickey AJ, Martonen TB. Fundamental effects of particle morphology on lung delivery: predictions of Stokes' law and the particular relevance to dry powder inhaler formulation and development. Pharm Res 2002; 19: 239–245.

Dahl R, Chung FK, Buhl R, Magnussen H, Nonikov V, Jack D, Bleasdale P, Owen R, Higgins M, Kramer B, on behalf of the INVOLVE. Efficacy of a new once-daily long-acting inhaled β2-agonist indacaterol versus twice-daily formoterol in COPD. Thorax 2010; 65:473-479.

Darquenne C. Aerosol deposition in the human respiratory tract breathing air and 80:20 heliox. J Aerosol Med 2004; 17: 278-285.

Das S, Larson I, Young PM, Stewart P. Agglomerate properties and dispersibility changes of salmeterol xinafoate from powders for inhalation after storage at high relative humidity. Eur J Pharm Sci 2009; 37: 442-450.

Desai TR, Hancock REW, Finlay WH. Delivery of liposomes in dry powder form: aerodynamic dispersion properties. Eur J Pharm Sci 2003; 20: 559–567.

Dinesh BS, Gleb BS. Engineered microcrystals for direct surface modification with layer-by-layer technique for optimized dissolution. Eur J Pharm Biopharm 2004; 58:521-527.

Driessen B. Pain: Systemic and Local/Regional Drug Therapy. Clin Tech Equine Pract 2007; 6: 135-144.

European Pharmacopoeia (Ph.Eur. 7.2). Preparations for Inhalation. Aerodynamic Assessment of Fine Particles—Fine Particle Dose and Particle Size Distribution (Ph. Eur. Method 2.9.18). 2012; 274-287.

FDA (CDER). Draft Guidance for Industry Metered Dose Inhaler (MDI) and Dry Powder Inhaler (DPI) Drug Products. 1998; CMC Documentation.

Forster A., Hempenstall J., Rades T. Investigation of drug/polymer interaction in glass solutions prepared by melt extrusion. Internet Journal of Vibrational Spectroscopy 2001; 5: 2-6.

Frust DE. Meloxicam: Selective COX-2 inhibition in clinical practice. Seminars in Arthritis and Rheumatism 1997; 26:21-27.

Gainotti A, Bettini R, Gazzaniga A, Colombo P, Giordano F. Drug-beta-cyclodextrin containing pellets prepared with a high-shear mixer. Drug Dev Ind Pharm 2004; 30:1061-1068.

Geller DE, Konstan MW, Smith J, Noonberg SB, Conrad C. Novel tobramycin inhalation powder in cystic fibrosis subjects: Pharmacokinetics and safety. Pediatr Pulm 2007; 42: 307–313.

Gerrity TR, Garard CS, Yeates DB. A mathematical model of particle retention in the air-spaces of human lungs. Brit J Indust Med 1983; 40:121-130.

Ghofrani HA, Osterloh IH, Grimminger F. Sildenafil: from angina to erectile dysfunction to pulmonary hypertension and beyond. Nat Rev Drug Discov 2006; 5:689-702.

Glover W, Chan HK, Eberl S, Daviskas E, Verschuer J. Effect of particle size of dry powder mannitol on the lung deposition in healthy volunteers. Int J Pharm 2008; 349: 314–322.

Goldman AP, Williams CS, Sheng H, Lamps LW, Williams VP, Pairet M, Morrow JD, DuBois RN. Meloxicam inhibits the growth of colorectal cancer cells. Carciogenesis 1998; 19:2195-2199.

Gonda I. The ascent of pulmonary drug delivery. J Pham Sci 2000; 89: 940-945.
Hassan HB, Kata M, Erős I, Aigner Z. Preparation and Investigation of Inclusion Complexes Containing Gemfibrozil and DIMEB. J Incl Phenom 2004; 50:219-226.
Hetzel MR, CLARK TJH. Comparison of salbutamol Rotahaler with conventional pressurized aerosol. Clin Exp Allergy 1977; 7: 563-568.
Heyder J, Gebhart J, Rudolf G, Schiller CF, Stahlhofen W. Deposition of particles in the human respiratory tract in the size range 0.005-15. J Aerosol Sci 1986; 17: 811-825.
Hickey AJ. Inhalation aerosol- Physical and biological basis for therapy. 2006; Vol.94, Marced Dekker Ed. New York.
Hindle M, Makinen G. Effects of humidity on the in vitro aerosol performance and aerodynamic size distribution of cromolyn sodium for inhalation. Eur J Pharm Sci 1996; 4: S142.
Hinds WC. Aerosol Technology. 1999; John Wiley & Sons, Ltd. New York.
Hulse WL, Forbes RT, Bonner MC, Getrost M. The characterization and comparison and spray dried mannitol samples. Drug Dev Ind Pharm 2009; 35: 712-718.
ICRP Publication. Human Respiratory Tract Model for radiological protection. 1994; Elsevier Science 24: 1-120.
Inhalanda. Preparations for inhalation. Pharm Eur Suppl 1998; 0671: 984–989.
Islam N, Gladki E. Dry powder inhalers (DPIs)—A review of device reliability and innovation. Int J Pharm 2008; 30: 1.
Jafar M, Mhg D, Shareef A. Enhancement of dissolution and anti-inflammatory effect of meloxicam using solid dispersion. Int J Appl Pharm 2010; 2: 22-27.
Kaerger SJ, Price R, Young PM, Tobym MJ. Carriers for DPIs: formulation and regulatory challenges. Pharmaceutical Technology Europe 2006; October edition: 25-30.
Kaialy W, Nokhodchi A. Treating mannitol in a saturated solution of mannitol: a novel approach to modify mannitol crystals for improved drug delivery to the lungs. Int J Pharm 2013; 448: 58–70.
Kaialy W, Nokhodchi A. Freeze-dried mannitol for superior pulmonary drug delivery via dry-powder inhaler. Pharm Res 2013; 30: 458–477.
Kaialy W, Larhrib H, Ticehurst MD, Nokhodchi A. Influence of batch cooling crystallization on mannitol physical properties and drug disposition from dry-powder inhalers. Cryst Growth Des 2012; 3006–3017.
Nokhodchi A, Larhrib H. Overcoming the undesirable properties of dry-powder inhalers with novel engineered mannitol particles. Ther Deliv 2013; 4: 879-882.
Khan KA. The concept of dissolution efficiency. J Pharm Pharmacol 1975; 27: 48-49.
Khassawneh BY, Al-Ali MK, Alzoubi KH, Batarseh MZ, Al-Safi SA, Sharara AM, Alnasr HM. Handling of inhaled devices in actual pulmonary practice: metered-dose inhaler versus dry powder inhalers. Respir Care 2008; 53: 324-328.
Koebrich R, Rudolf G, Stahlhofen W. A mathematical model of mass deposition in man. Ann Occup Hyg 1994; 38: 15-23.
Labiris NR, Dolovich MB. Pulmonary drug delivery. Part II: The role of inhalant delivery devices and drug formulations in therapeutic effectiveness of aerosolized medications. ‚J Clin Pharmacol 2003; 56: 600-612.
Laube BL, Benedict GW, Dobs AS. Time to peak insulin level, relative bioavailability and effect of site of deposition of nebulized insulin in patient with noninsulin-dependent diabetes mellitus. J Aerosol Med - Deposition Clearance and Effects in the Lung 1998; 11: 153-173.
Le Brun PH, De Boer A, Heijerman HGM, Frijlink HW, A review of the technical aspects of drug nebulisation. Pharm World Sci 2000; 22: 75-81.
Le VN, Robins E, Flament MP. Agglomerate behaviour of fluticasone propionate within dry powder inhaler formulations. Eur J Pharm biopharm. 2012; 80: 596-603.
Li HY, Seville PC, Williamson IJ, Birchall JC. The use of amino acids to enhance the aerolisation of spray-dried powders for pulmonary gene therapy. J Gene Med. 2005; 7: 343–353.
Lippmann M, Yeates DB, Albert RE, Deposition, retention and clearance of inhaled particles. Brit J Ind Med 1980; 37: 337-362.
LiCalsi C, Christensen T, Bennett JV, Phillips E, Whitman C. Dry powder inhalation as a potential delivery method for vaccines. Vaccine 1999; 17: 1796-1803.
Lida K, Hayakawa Y, Okamoto H, Danjo K, Luenberger H. Influence of storage humidity on the in vitro inhalation properties of salbutamol sulphate dry powder with surface covered lactose carrier. Chem Pharm Bull 2004; 52: 444–446.
Li Q, Rudolph V, Weigl B, Earl A. Interparticle van der Waals force in powder flowability and compactibility. Int J Pharm 2004; 280: 77–93.
Lippmann M. Effects of fiber characteristic on lung deposition, retention and disease. Env Health Persp 1990; 88: 311-217.
Lipinski CA. Drug-like properties and the causes of poor solubility and poor permeability. J Pharmacol Toxicol Methods 2000; 44: 235-249.

Luger P, Daneck K, Engel W, Trummlitz G, Wagner K. Stucture and physicochemical properties of meloxicam, a new NSAID. Eur J Pharm Sci 1996; 4:175-187.

Mallet JP, Diot P, Lemarie E. Aerosols for administration of systemic drugs. Rev Mal Respir. 1997; 14: 257-267.

Mao L, Blair J. Effect of additives on the aerolisation properties of spray dried trehalose powders. Resp Deliv Drugs 2004; 9: 653–656.

Moren F, Dolovich M, Newhouse M, Newman S. Aerosols in Medicine: Principles, Diagnosis and Therapy. 1993; Elsevier Science Publishers. Amsterdam.

Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983; 65: 55–63.

Naidu NB, Chowdary KPR, Murthy KVR, Satyanarayana V, Hayman AR, Becket G. Physicochemical characterization and dissolution properties of meloxicam–cyclodextrin binary systems. J Pharm Biomed Analysis 2004; 35:75–86.

Nassab PR, Rajkó R, Szabó-Révész P. Physicochemical characterization of meloxicam–mannitol binary systems. J Pharm Biomed Anal 2006; 41:1191-1197.

Newman SP, Clarke SW. Therapeutic aerosol 1 – Physical and practical considerations. Torax 1983; 38: 881-886.

Newman SP. Principles of metered-dose inhaler design. Res.Care 2005; 50 (9): 1177-1190.

Nora YK, Chan HK. *In Vitro* Aerosol Performance and Dose Uniformity between the Foradile® Aerolizer® and the Oxis® Turbuhaler® J Aerosol Med. 2001; 14: 495-501.

Oberoi LM, Kenneth AS, Riga AT. Study of interaction between ibuprofen and nicotinamide using differential scanning calorimetry, spectroscopy, and microscopy and formulation of a fast-acting and possibly better ibuprofen suspension for osteoarthritis patients. J Pharm Sci 2005; 94:93-101.

O'Rahilly R. Basic Human Anatomy. 1983; W.B. Saunders. Philadelphia.

Paudel A, Worku ZA, Meeus J, Guns S, Van den Mooter. Manufacturing of solid dispersion of poorly water soluble drugs by spray drying: Formulation and process considerations. Int J Pharm 2013; 453: 253-284.

Parlati C. Respirable microparticles of aminoglycoside antibiotics for pulmonary. PhD thesis. Department of pharmaceutical technology, Parma. 2008.

Patton JS: Mechanism of macromolecule absorption by the lungs. Adv Drug Deliver Rev 1996, 39: 3-36.

Patton JS, Byron PR. Inhaling medicines: delivering drugs to the body through the lungs. Nat Rev Drug Discov 2007; 67-74.

Pilcer G, Amighi K. Formulation strategy and use of excipients in pulmonary drug delivery. Int J Pharm 2010; 392: 1–19.

Possmayer F, Nag K, Rodriguez K, Qanbar R, Schürch S. Surface activity in vitro: role of surfactant proteins. Comp Biochem Phys 2001; 129: 209–220.

Prime D, Atkins PJ, Slater A, Sumby B. Review of dry powder inhalers. Adv Drug Deliver Rev. 1997: 51-58.

Prota L, Santoro A, Bifulco M, Aquino RP, Mencherini T, Russo P. Leucine enhances aerosol performance of Naringin dry powder and its activity on cystic fibrosis airway epithelial cells. Int J Pharm 2011; 412: 8–19.

Rabbani NR, Seville PC. The use of amino acids as formulation excipients in lactose based spray-dried powders. J Pharm Pharmacol 2004; 56: 32–33.

Rabinowitz JD, Zaffaroni AC. Delivery on nonsteroidal antiinflammatory drugs through an inhalation route. United States Patent 2004; 6716417.

Robinson M, Daviskas E, Eberl S, Baker J, Chan HK, Anderson SD, Bye PT. The effect of inhaled mannitol on bronchial mucus clearence in cystic fibrosis patients: a pilot study. Eur Respir J 1999; 14: 678–685.

Robinson BV, Sullivan FM, Borzelleca JF, Schwartz SL. PVP – A Critical Review of the Kinetics and Toxicology of Polyvinylpyrrolidone (Povidone). 1st 1990; Lewis Publishers, Inc. Chelsea.

Rowe RC, Sheskey PJ, Weller PJ. Pharmaceutical Excipients. 2001; Pharmaceutical Press, London.

Sakagami M, Kinoshita W, Sakon K, Sato J, Makino Y. Mucoadhesive beclomethason dipropionate microsheres for powder inhalation: their pharmacokinetics and pharmacodynamic evaluation. J Control Release 2002; 80: 207-218.

Sanna V, Kirschvink N, Gustin P, Gavini E, Roland I, Delattre L, Evrard B. Preparation and in vivo toxicity study of solid lipid microparticles as carrier for pulmonary administration. AAPS Pharm Sci Technol 2004; 5: 27–32.

Sauret V, Halson PM, Brown IW, Fleming JS, Bailey AG. Study of the three-dimensional geometry of the central conducting airways in man using computed tomographic (CT) images, J Anat 2002; 200: 123-34.

Scheuch G, Stahlhofen W. Particle deposition of inhaled aerosol boluses in the upper human airways. J Aerosol Sci 1987; 18: 725-727.

Schulz H, Brand P, Heyder J. Particle deposition in the respiratory tract. 2000; Marcel Dekker, New York.

Seville PC, Learoyd TP, Li HY, Williamson IJ, Birchall JC. Amino acid modified spray dried powders with enhanced aerosolisation properties for pulmonary drug delivery. Powder Technol 2007; 178: 40–50.

Shoyele SA. Controlling the release of proteins/peptides via the pulmonary route. Methods Mol Biol 2008; 437: 141–148.

Shou-Cang S, Kiong W NG, Chia L, Dong YC, Reginald BH. In Press. Stabilized Amorphous State of Ibuprofen by Co-Spray Drying With Mesoporous SBA-15 to Enhance Dissolution Properties. J Pharm Sci 2010; 99: 1997-2007.

Smith IJ, Parry-Billings M. The inhalers of the future? A review of dry powder devices ont he market today. Pulm Pharmacol Ther. 2003; 79-95.

Souza RF, Shewmake K, Beer DG, Cryer B, Spechler SJ. Selective inhibition of cyclooxygenase-2 suppresses growth and induces apoptosis in human oesophageal adenocarcinoma cells. Cancer Res 2005; 60: 5767–5772.

Steckel H, Brandes HG. A novel spray-drying technique to produce low density particles for pulmonary delivery. Int J Pharm 2004; 278: 187–195.

Steckel H, Bolzen N. Alternative sugars as potential carriers for dry powder inhalations. Int J Pharm 2004; 270: 297–306.

Stuart BO. Deposition and clearance of inhaled particles. Env.Health Persp 1984; 55: 369-390.

Tamaoki J, Nakata J, Nishimura K, Kondo M, Aoshiba K, Kawatani K, Nagai A. Effect of inhaled indomethacin in asthmatic patients taking high doses of inhaled corticosteroids. J Allergy Clin Immunol 2000; 105: 1134-1139.

Tee SK, Marriott C, Zeng XM, Martin GP. The use of different sugars as fine and coarse carriers for aerosolized salbutamol sulphate. Int J Pharm 2000; 208: 111–123.

Telko MJ, Hickey AJ. Dry powder inhaler formulation. Respir Care 2005; 50: 1209–1227.

Traini D, Scalia S, Adi H, Marangoni E, Young PM. Polymer coating of carrier excipients modify aerosol performance of adhered drugs used in dry powder inhalation therapy. 2012; 438: 150-159.

Tsubouchi Y, Sano H, Yamada R, Hashiramoto A, Kohno H, Kusaka Y, Kondo H. Preferential inhibition of cyclooxygenase-2 by Meloxicam in human rheumatoid synoviocytes. Eur J Pharmacol 2000; 395:255-263.

Tsubouchi Y, Mukai S, Kawahito Y, Yamada R, Kohno M, Inoue KI, Sano H. Meloxicam inhibits the growth of non small cell lung cancer. Anticancer Res 2000; 20: 2867–2872.

Van Noord JA,. De Munck DRAJ, Bantje TA, Hop WCJ, Akveld MLM, Bommer AM. Long-term treatment of chronic obstructive pulmonary disease with salmeterol and the additive effect of ipratropium. Eur Respir J 2000; 15: 878-885.

Vaugh JM, Wiederhold NP. Murine airway histology and intracellular uptake of inhaled amorphous itraconazole. Int J Pharm 2007, 338: 219-224.

Veinhard R. Pharmaceutical particle engineering via Spray Drying. Pharm Res 2008; 25: 999-1022.

Weibel E. Morpometry of the Human Lung. 1963; Springer Verlag. Berlin.

Wetterlin K. Turbohaler-a new powder inhaler for administration of drugs to the airways. Pharm Res. 1988;5: 506-508.

Weyna DR. Crystal engineering of multiple component crystal forms active pharmaceutical ingredients. PhD thesis. Department of chemistry College of Arts and Sciences, Florida, 2011.

Wong W, Crapper J, Chan HK, Traini D, Young PM. Pharmacopeial methodologies for determining aerodynamic mass distribution of ultra-high dose inhalers medicines. J Pharm Biomed Anal 2010; 51: 853–857.

Woolfe AJ, Zeng XM, LAngford A. Method to produce powders for pulmonary or nasal administration. 2001; WO patent 0113885. 2001.

Wu S. Calculation of interfacial tension in polymer systems, J Polym Sci 1971; 34: 19-30.

Yeh HC, Phalen RF, Raabe OG. Factors influencing the deposition of inhaled particles. Env Health Persp 1976; 15: 147-156.

Young PM, Traini D, Chan HK, Chiou H, Edge S, Tee T. The influence of mechanical processing dry powder inhaler carriers on drug aerosolisation performance. J Pharm Sci 2007; 96: 1331-1341.

Young PM, Price R, Tobyn M, Buttrum M, Dey F. Effect of humidity on aerolisation of micronized drugs. Drug Dev Ind Pharm 2003; 29: 959–966.

Young PM, Price R. The influence of humidity on the aerolisation of micronized and SEDS produced salbutamol sulphate. Eur J Pharm Sci 2004; 22: 235–240.

Zajca N, Obrezaa A, Beleb M, Srčič S. Physical properties and dissolution behaviour of nifedipine/mannitol solid dispersions prepared by hot melt method. Central European Symposium on Pharmaceutical Technology and Biotechnology, 2008; 291: 51-58.

Zeng X, MacRitchie H, Marriott C, Martin G. Humidity-induced changes of the aerodynamic properties of dry powder aerosol formulations containing different carriers. Int J Pharm 2007; 333: 45–55.

Zijlstra GS, Hinrichs WLJ, De Boer A, Frijlink HW. The role of particle engineering in relation to formulation and de-agglomeration principle in the development of a dry powder inhalation of cetrorelix, Eur J Pharm Sci 2004; 23: 139-149.

ZuWallack AR, ZuWallack RL. Tiotropium bromide, a new, once daily inhaled anticholinergic bronhodilatator for chronic-obstructive pulmonary disease. Expert Opin Pharmaco 2004; 5: 1827-1835.

[http://www.histology.leeds.ac.uk/respiratory/conducting.php]

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ANNEX

PUBLICATION I.

PUBLICATION II.

PUBLICATION III.

PUBLICATION IV.

PUBLICATION V.

PUBLICATION VI.