

University of Groningen

## A levodopa dry powder inhaler for the treatment of Parkinson's disease patients in off periods

Luinstra, Marianne; Grasmeyer, Floris; Hagedoorn, Paul; Moes, Jan Reindert; Frijlink, Henderik W.; De Boer, Anne H.

*Published in:*  
European Journal of Pharmaceutics and Biopharmaceutics

*DOI:*  
[10.1016/j.ejpb.2015.10.003](https://doi.org/10.1016/j.ejpb.2015.10.003)

**IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.**

*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2015

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Luinstra, M., Grasmeyer, F., Hagedoorn, P., Moes, J. R., Frijlink, H. W., & De Boer, A. H. (2015). A levodopa dry powder inhaler for the treatment of Parkinson's disease patients in off periods. *European Journal of Pharmaceutics and Biopharmaceutics*, 97, 22-29. <https://doi.org/10.1016/j.ejpb.2015.10.003>

### Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

### Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

*Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.*



## Research Paper

# A levodopa dry powder inhaler for the treatment of Parkinson's disease patients in off periods



Marianne Luinstra<sup>a,b,\*</sup>, Floris Grasmeijer<sup>a</sup>, Paul Hagedoorn<sup>a</sup>, Jan Reindert Moes<sup>b</sup>, Henderik W. Frijlink<sup>a</sup>, Anne H. de Boer<sup>a</sup>

<sup>a</sup> Department of Pharmaceutical Technology and Biopharmacy, University of Groningen, Ant. Deusinglaan 1, 9713 AV Groningen, The Netherlands

<sup>b</sup> Department of Clinical Pharmacy, Martini Hospital, Van Swietenplein 1, 9728 NT Groningen, The Netherlands

## ARTICLE INFO

## Article history:

Received 22 July 2015

Revised 1 October 2015

Accepted in revised form 3 October 2015

Available online 13 October 2015

## Keywords:

Levodopa

Parkinson's disease

Off period

Dry powder inhalation

Cyclops

## ABSTRACT

Adequate treatment of Parkinson's patients in off periods with orally administered levodopa is hindered by a poor bioavailability and a slow onset of action. Hence, there is a need for a fast and reliable alternative as for instance via pulmonary administration of the drug. We developed a levodopa containing powder formulation for pulmonary delivery by a recently presented high dose dry powder inhaler (Cyclops). The objective was to produce the drug formulation by means of simple techniques such as micronization, either as pure active substance or with a minimum amount of excipients. After an initial screening on dispersion behaviour, the most promising formulation in the Cyclops was characterized *in vitro* over a range of pressure drops (2–6 kPa) and doses (20, 30 and 40 mg), representative of those to be expected in practice. A co-micronized levodopa formulation with 2% L-leucine appeared to yield the best aerosol properties for inhalation and highest delivered dose reproducibility. The combination of this particular formulation and the Cyclops inhaler seems to meet the basic requirements for satisfactory deposition in the airways. This formulation is therefore expected to be a promising candidate for the treatment of Parkinson's patients in an off period.

© 2015 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 1. Introduction

Parkinson's disease is a progressive neurodegenerative disorder characterized by the degeneration of dopaminergic neurons in the substantia nigra. This causes a lack of dopamine in the striatum [1], which disrupts motor circuits in the brain and results in motor function impairments such as tremor, rigidity and bradykinesia [2].

Treatment of Parkinson's disease with dopaminergic drugs (especially levodopa) will improve motor function, but many patients develop motor fluctuations after several years of therapy as a result of a narrowing therapeutic window of levodopa [3]. Fluctuating plasma concentrations due to irregular gastric emptying and intermittent oral intake are characteristic for orally taken levodopa. They result in periods in which the symptoms of Parkinson's disease are well controlled ('on periods') alternated by poorly controlled ('off') periods [4]. Symptoms during off periods are not limited to decreased mobility, but also include sensory symptoms, autonomic symptoms and psychiatric disorders [5]. After subcutaneous apomorphine injection for the management of

motor fluctuations, an onset of effect is generally seen within 20 min [6], but because of the huge discomfort for the patient an even faster response is desired. The use of apomorphine also causes nausea and vomiting, which makes it necessary for patients to use anti-emetics on a regular base [7]. Additionally, the use of subcutaneous apomorphine requires (self) injection, which is an invasive administration technique that poses a burden to the patient. For all these reasons, there is a need for a more reliable, patient friendly and faster acting new treatment.

A promising alternative for the treatment of off periods is the pulmonary administration of levodopa. Bartus et al. [8] showed that an inhalable formulation of levodopa had superior pharmacokinetic properties in rats. After pulmonary delivery of 2 mg levodopa, plasma levels were elevated faster than after oral administration of the same dose, with times to reach peak values of  $4.7 \pm 1.9$  min and  $24 \pm 4.0$  min, respectively. Peak plasma levels were also considerably higher after pulmonary administration compared to oral administration of the same dose ( $4.8 \pm 1.1$  µg/mL versus  $1.8 \pm 0.40$  µg/mL).

A levodopa dry powder inhalation product is currently being developed by Civitas, now Acorda therapeutics (CVT-301 program). A phase IIa dose finding study [9,10] performed by Acorda therapeutics showed significant improvement in motor function in

\* Corresponding author at: Martini Hospital, Van Swietenplein 1, 9728 NT Groningen, The Netherlands. Tel.: +31 50 5246722.

E-mail address: [m.luinstra@mzh.nl](mailto:m.luinstra@mzh.nl) (M. Luinstra).

Parkinson's patients after pulmonary administration of levodopa in an off period. Doses tested were 25 and 50 mg and the phase IIb study showed that inhaled levodopa was also well tolerated [11]. Therefore, pulmonary administration of levodopa seems suitable for use as 'rescue' therapy in Parkinson's patients during off periods.

Despite the current development of Acorda therapeutics, there seems room for further improvement and optimization. Acorda therapeutics use the ARCUS<sup>®</sup> inhaler, which is a capsule based, breath-actuated device [12], with spray-dried levodopa particles. Such particles may have improved dispersion performance [13,14], but disadvantages include the increased volume of powder that needs to be inhaled [15], the use of several excipients that further increase the volume of the dose and the high price of the applied particle engineering process. Next to spray drying, milling is a commonly used technique for particle size reduction of inhalation powders. Depending on the type of mill and conditions applied, milling may result in a relatively broad particle size distribution for inhalation as well as in solid-state transformation (partial amorphization) and electrostatic charging of the powder particles [16–18]. This also depends on the type of drug; however, and despite these potential drawbacks, milling also has certain advantages over spray drying, since milling is simpler, cheaper and easier to scale up from development to production. Besides, spray drying generally yields fully amorphous powders, which are less stable and more moisture sensitive than micronized powders.

Using a simple and disposable, preloaded and high resistance, unit-dose inhaler such as the Cyclops has many advantages over classic capsule inhalers regarding ease of operation, oropharyngeal deposition and moisture uptake by particles retained in the inhaler [19]. Preparing the inhaler for inhalation is limited to pulling a strip of lidding foil, projecting from the rear end of the inhaler, from the blister cup (single step operation). Particularly for Parkinson's patients with impaired motor function these may be highly relevant aspects.

In this study, we aim to address the issues mentioned in the previous paragraphs by screening and evaluating spray dried and milled levodopa formulations with minimal use of excipients. For dispersion of the formulations, the newly developed Cyclops disposable inhaler for high drug doses was used [19].

## 2. Materials and methods

### 2.1. Study design for the development of a potentially suitable levodopa formulation

In this study, we started inhaler dispersion experiments with pure micronized and spray dried levodopa to obtain simple and cheap to produce powders. We also added small amounts of L-leucine to the drug before micronization and spray drying to investigate whether this brings improvement in dispersion and inhaler retention. For a fast screening of the suitability of these powders for inhalation with the Cyclops we used laser diffraction technique to compare the particle size distribution (PSD) in the aerosol with the PSD of the primary particles from RODOS dispersion (3 bar), which enables assessment of the dispersion efficiency of the inhaler-formulation combination. Only for the most promising candidate formulation from the laser diffraction analysis screening, cascade impactor experiments were conducted.

### 2.2. Starting materials and Cyclops inhaler

Levodopa, Ph Eur quality, was purchased from Duchefa Farma (Haarlem, the Netherlands). Levodopa > 98% and L-leucine were

supplied by Sigma–Aldrich (Zwijndrecht, the Netherlands). L-leucine is a hydrophobic amino acid which is endogenous to the lungs [20].

The air classifier based Cyclops inhaler used in this study for dispersion of the formulations was a machined prototype and has been described in detail before [19]. In brief, the Cyclops has a plate-like design and the inhaler makes use of air classifier technology for dispersion of the powder. The Cyclops contains an aluminium blister as dose compartment. Micronized or spray dried powders were weighed manually into the blisters immediately before the dispersion experiments (amounts referred to as metered dose). Metered doses were 20, 30 and 40 mg. The Cyclops is a high resistance device ( $0.060 \text{ kPa}^{0.5} \text{ min L}_N^{-1}$ ) and flow rates corresponding to 2, 3, 4 and 6 kPa are 24, 29, 34 and 44  $\text{L}_N/\text{min}$  respectively.

### 2.3. Powder preparation by (co-)micronization

Two different qualities of levodopa (Duchefa and Sigma–Aldrich) were investigated in this study. Levodopa and L-leucine were mixed manually in a glass beaker for 60 s prior to (co-) micronization in small batches of 2 g using a spatula. Different concentrations of L-leucine (0, 1, 2, 5 and 6.4% w/w) were used. Pure levodopa and the mixtures of levodopa with L-leucine were micronized using a 50 AS jet mill (Alpine Hosokawa, Germany). The applied nozzle and milling pressures for water free nitrogen (as milling gas) of 6 and 2 bar respectively and the feed rate of the mill (by spatula) were used to control the size distribution of the powder within pre-set values for the median diameter ( $X_{50}$ :  $1.10 \mu\text{m} < X_{50} < 1.50 \mu\text{m}$ ) and FPF  $< 5 \mu\text{m}$  ( $> 80\%$ ). Batch sizes after micronization were approximately 1 g and multiple batches of each type of formulation were prepared to complete all measurements. Milled levodopa formulations were stored at room temperature in closed glass vials without further specific precautions taken.

In addition to micronization, also spray drying of levodopa was explored as powder preparation technique. However, levodopa is instable in aqueous solution [25] and spray-dried levodopa also appeared to exhibit a rapid moisture uptake after brief exposure to ambient air, which resulted in poor dispersion and high inhaler retention. Therefore, it was decided in an early phase of the study not to continue with spray-dried levodopa.

### 2.4. Characterization of the starting materials and the formulations

#### 2.4.1. Scanning electron microscopy (SEM)

In order to determine the shape and surface texture (roughness) of the starting materials and different (co-)micronized levodopa–leucine combinations, scanning electron microscopy was performed with a JEOL 6301F microscope (Jeol, Japan). An acceleration voltage of 3 kV was used. All samples were sputter coated with 10 nm of a gold/palladium alloy.

#### 2.4.2. Laser diffraction analysis (LDA)

The particle size distributions of the starting materials and various powder mixtures were measured with a Sympatec HELOS BF laser diffractometer (Sympatec GmbH, Clausthal-Zellerfeld, Germany). The samples were dispersed in the laser beam with a RODOS (Sympatec) disperser at 0.5; 3 and 5 bar. Only the 3 bar data are shown, because the PSDs appeared to be the same at 3 and 5 bar, indicating that all particles are primary entities, whereas no particle breakup occurs at 3 bar. The diffractometer was equipped with a 100 mm lens (R3). The Fraunhofer theory was used for computation of the particle size distributions from the complex diffraction patterns. Each sample was measured twice at each dispersion condition.

#### 2.4.3. Dynamic vapour sorption (DVS) analysis

Dynamic vapour sorption analysis was performed with the DVS 1000 (Surface Measurement Systems Limited, United Kingdom) to determine the sorption and desorption (hygroscopicity) of the starting materials and some selected drug–excipient combinations. Moisture isotherms were collected at 25 °C from 0% to 90% relative humidity (RH) in steps of 10% RH after previous drying of the sample at 0% RH. The next target RH was first set when the sample mass change ( $dm/dt$ ) decreased to  $\leq 0.0005\%/min$ .

#### 2.4.4. Differential scanning calorimetry (DSC)

Differential scanning calorimetry was used to collect information about the solid state properties of the levodopa samples before and after micronization. DSC was performed with a DSC Q2000 (TA Instruments, Ghent, Belgium). Samples of 2–6 mg were weighed in open aluminium pans and heated from 0 to 290 °C at a rate of 20 °C per minute.

#### 2.5. Determination of the range of dose weights for *in vitro* testing

The dispersion efficacy of an inhaler for a specific powder formulation depends among other things on the dose weight to be administered. Therefore, development and testing of formulations for a particular type of inhaler have to be conducted for the dose weight expected for the final product. Oral levodopa is delivered in a dose range of 25–100 mg, depending on the amount needed to terminate an off period. However, improved bioavailability can be expected from inhalation. Because the final dose is uncertain in the development phase, we tested a range of dose weights based on the finding of Bartus et al. [8] that 2–3 times higher maximum plasma levels are reached in rats after pulmonary administration compared to oral administration ( $4.8 \pm 1.1 \mu\text{g/mL}$  versus  $1.8 \pm 0.4 \mu\text{g/mL}$ ). Acorda therapeutics [9] showed that a dose in a range from 25 to 50 mg inhaled dose is effective in improving the Unified Parkinson's disease rating Section III motor score, which is a primary outcome measure in most clinical trials of Parkinson's disease therapeutics [21]. Knowing from previous experiments that the Cyclops is a highly efficient inhaler, we decided to limit the range of dose weights to 20–40 mg. Because we are aiming for use as 'rescue' therapy during off episodes next to standard oral maintenance therapy with levodopa plus a decarboxylase inhibitor, there is no need to add a decarboxylase inhibitor to the pulmonary formulation.

#### 2.6. Aerosol characterization from the Cyclops inhaler

##### 2.6.1. Laser diffraction analysis (LDA)

A special inhaler adapter Inhaler 2000™ (Sympatec) was used to connect the Cyclops with the laser diffraction apparatus [22], which was the same as used for characterization of the starting materials. Also the same 100 mm (R3) lens and Fraunhofer approximation theory were used for a direct comparison of the PSDs of the powders prepared and that of the aerosols from the inhaler. A fixed pressure drop (2, 3, 4 or 6 kPa) across the Cyclops during dispersion experiments was applied for the duration of three seconds. The number of replicate measurements ranged from 2 to 8, depending on the type of formulation and the dose weight.

##### 2.6.2. Time sliced measurements

The same laser diffractometer and conditions as described in the previous paragraph were used for time sliced measurements in order to follow the aerosol emission rate from the inhaler on the basis of the optical concentration in the laser beam. Sliced measurements of 0.1 s were performed to a total time of 5 s. The measurements were performed in duplicate. Complete emission

of the dose was assumed when the optical concentration reached the baseline.

##### 2.6.3. Cascade impaction analysis (CIA)

The aerodynamic PSDs of the levodopa aerosols from the Cyclops were measured with CIA using the Next Generation Impactor (NGI: Copley Scientific, United Kingdom), only for co-micronized levodopa with 2% L-leucine as most promising formulation. On the stages 2–7 glass fibre filters were added which were soaked with 1.5 mL water to reduce bounce effects [23]. In contrast to the laser diffraction measurements, for the NGI experiments, the inhalation times corresponding to an inhaled volume of 4 L were calculated for the flow rates corresponding to pressure drops of 2, 3, 4 and 6 kPa across the Cyclops. Cutoff diameters of the impactor stages were calculated with the equations described in the European Pharmacopoeia, 8th edition [24]. Measurements were performed in duplicate.

##### 2.6.4. Consistency of delivered dose

Determination of the consistency of delivered dose was performed using the method described in the European Pharmacopoeia, 8th edition [24]. The applied pressure drop over the inhaler was 4 kPa and the inhalation time was chosen such that an inhaled volume of 4 L was reached. The metered dose was 30 mg micronized levodopa with 2% L-leucine for all 10 measurements performed.

##### 2.6.5. Sample analysis

Samples from CIA and uniformity of delivered dose testing were dissolved in water and analysed spectrophotometrically at a wavelength of 280 nm (Unicam UV 500, ThermoSpectronic, United Kingdom) after it was confirmed that L-leucine did not interfere with the absorbance measured at a wavelength of 280 nm. Calibration curves were constructed based on aqueous solutions of the levodopa starting material ( $R^2 = 0.9998$ , concentration range: 0.003–0.1 mg/mL). When needed, samples were passed through 0.2  $\mu\text{m}$  cellulose acetate filters to remove glass fibres from the filters used for the *in vitro* deposition and consistency of delivered dose testing.

##### 2.6.6. Inhaler retention

Inhaler retention was determined gravimetrically with an analytical balance after laser diffraction measurements and chemically (see Section 2.6.5) after CIA. Inhaler retention is the fraction of the dose that is retained in the blister and inhaler after dispersion (i.e. the non-emitted dose fraction).

##### 2.6.7. Definitions

FPF% refers to the fine particle fraction  $<5 \mu\text{m}$ , expressed as per cent of the metered dose.

The diameters  $X_{10}$ ,  $X_{50}$  and  $X_{90}$  correspond to the 10%, 50% and 90% cumulative volume per cent values of the cumulative volume distribution curve as function of the particle diameter from laser diffraction.

$D_{50}$  represents the 50% value of the cumulative mass distribution curve as function of the aerodynamic particle diameter (obtained from cascade impactor analysis) and equals the mass aerodynamic diameter (MMAD) of the fine particle fraction  $<5 \mu\text{m}$ .

### 3. Results and discussion

#### 3.1. Characterization of the starting materials

RODOS dispersion data showed that levodopa purchased from Sigma consists of larger particles than levodopa from Duchefa.

Because it is known that the PSD of the starting material may influence the micronization process and thus, cause a difference in PSD between products after micronization, both starting materials were investigated to study the robustness of the powder preparation technique.

SEM observation of the unm micronized levodopa samples of both Sigma–Aldrich and Duchefa suggested that the starting materials are crystalline (Fig. 1A for the Sigma–Aldrich sample). This was confirmed with the DSC data (Online supplement Fig. S1). Fig. 1B shows that L-leucine has a plate like structure. Dynamic vapour sorption testing of levodopa from Sigma–Aldrich showed an increase in mass of only 0.075% between 0% and 90% relative humidity. This small change in mass indicates that the starting material levodopa used, is a non-hygroscopic powder when it is in the crystalline state. Therefore, no special precautions with respect to moisture sorption during storage were taken for the levodopa starting material.

### 3.2. Characterization of the levodopa powders for inhalation

The primary (laser diffraction) particle size distributions of the different micronized powder batches are comparable for the Sigma and Duchefa levodopa batches and seem not influenced by the difference between the PSDs of the starting materials (Table 1). The amount of L-leucine does not seem to have an effect on the primary particle size distribution of the micronized powders. The small variations in PSD of the micronized products are primarily the result of differences in feeding rate to the laboratory scale fluid jet mill and differences between duplicate experiments are of the same order of magnitude as those between different powders. Feeding of the mill is manual and, therefore, not reproducible to the extreme. All micronized products met the pre-set values (specs) for  $X_{50}$ .

After micronization, levodopa showed an increase of 0.543% in mass between 0% and 90% relative humidity. This mass increase is somewhat larger than the increase found for the starting material and it can at least partly be explained by the increase in specific surface caused by the milling. Further, micronized pure levodopa showed no dramatic change in dispersion after exposure to 75% RH and 0% RH for three days. SEM images of micronized and co-micronized levodopa are shown in Fig. 2.

### 3.3. Dispersion of pure micronized levodopa in the Cyclops

Pure micronized levodopa was dispersed in the Cyclops at 4 kPa in dose weights of 20, 30 and 40 mg. Fig. 3A shows that the FPF <5  $\mu\text{m}$  as per cent of the metered dose was only approximately

**Table 1**

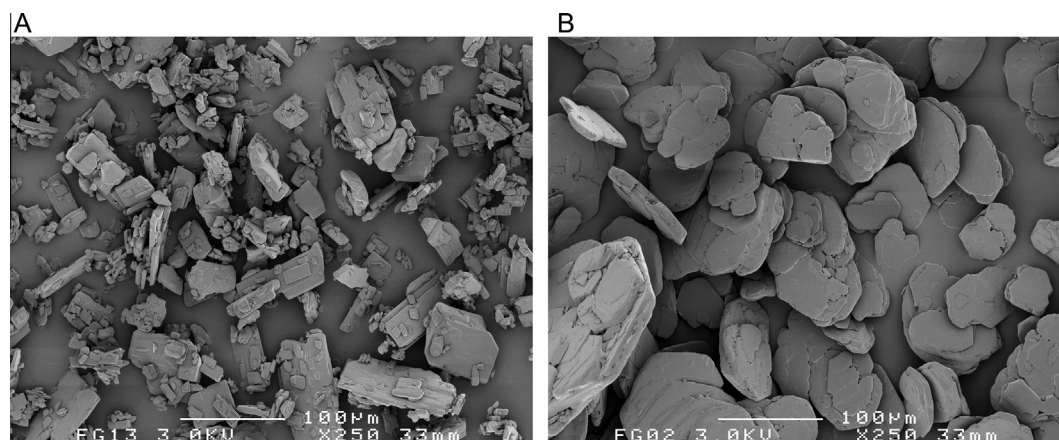
Mean  $X_{10}$ ,  $X_{50}$  and  $X_{90}$  values and the volume fractions <5  $\mu\text{m}$  (FPF <5  $\mu\text{m}$ ) obtained from the cumulative volume distribution curves of pure micronized levodopa and levodopa co-micronized with L-leucine,  $n = 2$ . D = Duchefa, S = Sigma.

Batch	Supplier	% L-leucine	$X_{10}$ ( $\mu\text{m}$ )	$X_{50}$ ( $\mu\text{m}$ )	$X_{90}$ ( $\mu\text{m}$ )	<5 $\mu\text{m}$ (%)
1	D	0	0.66	1.46	3.06	99.77
2	D	1	0.62	1.19	2.54	98.85
3	D	1	0.65	1.45	84.22	83.57
4	D	2	0.61	1.13	2.34	100.00
5	S	2	0.61	1.12	2.31	100.00
6	D	2	0.62	1.22	3.16	92.37
7	D	2	0.66	1.44	15.46	89.82
8	S	2	0.64	1.29	2.86	97.41
9	D	2	0.63	1.29	3.76	90.52
10	S	2	0.62	1.18	2.76	94.88
11	S	2	0.63	1.24	2.57	100.00
12	D	5	0.63	1.24	2.82	96.16
13	S	5	0.62	1.21	3.03	92.58
14	D	6.4	0.62	1.20	2.55	99.96

20–30% for all three doses tested. The mean  $X_{50}$ -values of the aerosols from laser diffraction technique were 2.9  $\mu\text{m}$ , 3.8  $\mu\text{m}$  and 4.1  $\mu\text{m}$  for the 20, 30 and 40 mg doses respectively, showing that the dose indeed affects dispersion efficiency (Fig. 3B). These median particle diameters in the aerosol are quite large compared to those of the primary particles being 1.46  $\mu\text{m}$  (see Table 1). This may be expected because the dispersion with RODOS (3 bar, equals 300 kPa) is at a much higher pressure than that with the Cyclops (4 kPa) and this results in the delivery of part of the dose from the inhaler as small agglomerates. Nevertheless, the FPF <5  $\mu\text{m}$  increased with increasing metered dose (Fig. 3A) and this can be explained with the decreasing inhaler retention from 70% (for the 20 mg dose) to 50% for the 30 and 40 mg dose. Although the Cyclops has a high resistance to air flow and, for that reason limits the inspiratory flow rate and the chance of oropharyngeal deposition of particles in the size range between 3 and 5  $\mu\text{m}$ , a better dispersion efficiency is preferred to obtain a high total lung deposition. Therefore, and to reduce the inhaler retention, we decided that pure micronized levodopa is not suitable for inhalation with the Cyclops and we continued with levodopa co-micronized with L-leucine.

### 3.4. The effect of L-leucine on the dispersion of micronized levodopa in the Cyclops

Co-micronization of levodopa caused a considerable increase in FPF <5  $\mu\text{m}$  as per cent of the metered dose, from 20–30% for pure



**Fig. 1.** SEM images of (A) levodopa unm micronized (250 $\times$ ); (B) L-leucine unm micronized (250 $\times$ ).

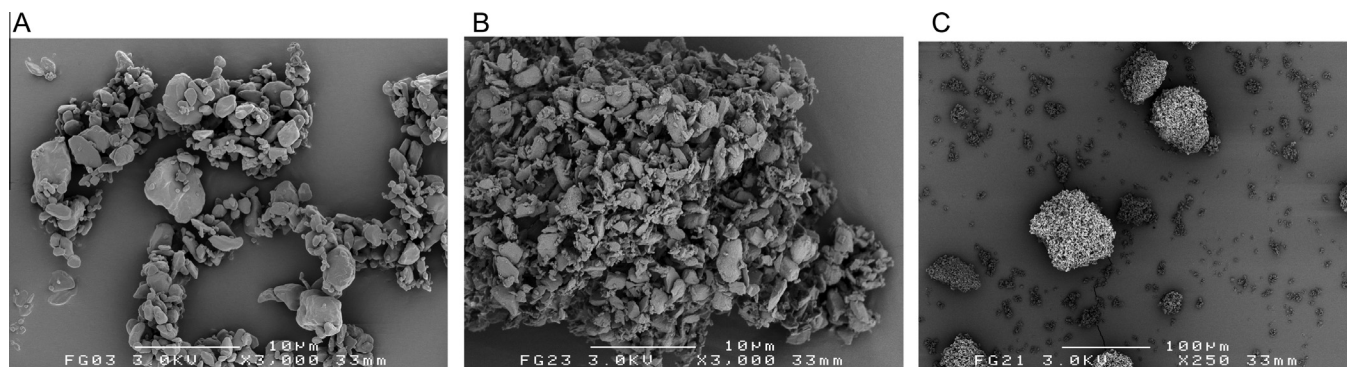


Fig. 2. SEM images of (A) micronized levodopa (3000 $\times$ ), (B) co-micronized levodopa and (2%) L-leucine (3000 $\times$ ), (C) co-micronized levodopa and (2%) L-leucine (250 $\times$ ).

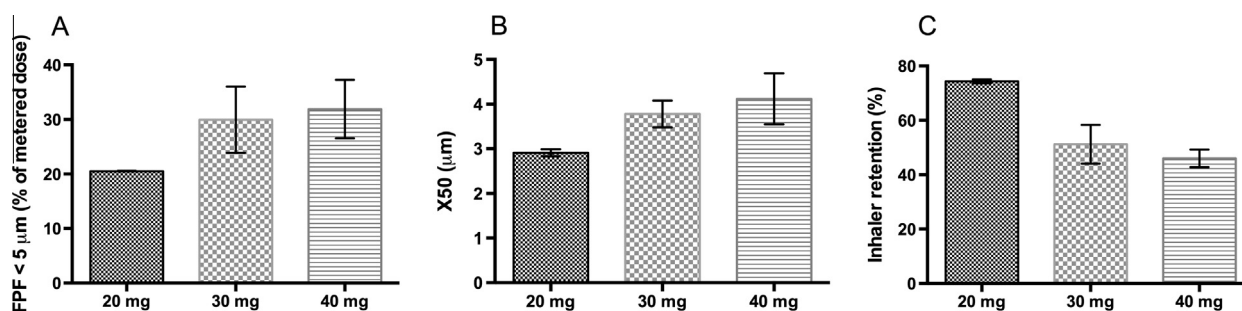


Fig. 3. Effect of levodopa dose on (A) FPF < 5  $\mu\text{m}$  as % of the metered dose, (B) X<sub>50</sub>-value of the aerosol from the Cyclops, (C) inhaler retention for pure micronized levodopa at 4 kPa. Mean and min-max values are shown.

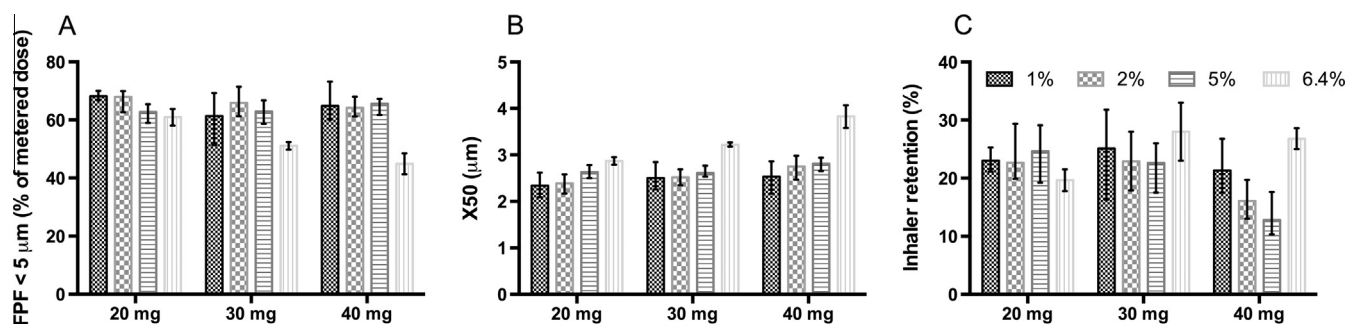


Fig. 4. Effect of mass % L-leucine in levodopa/L-leucine formulations on (A) FPF < 5  $\mu\text{m}$  as % of the metered dose, (B) X<sub>50</sub>-value of the aerosol from the Cyclops, (C) inhaler retention at 4 kPa. Mean and min-max values are shown.

levodopa to 60–70% for the mixture with only 1% L-leucine (Fig. 4A). Similar improvements of the FPFs compared to pure levodopa were seen for powders with 2% and 5% L-leucine. The increase in FPF is reflected by a lower X<sub>50</sub>-value for the mixtures with 1%, 2% and 5% L-leucine (Fig. 4B) compared to pure levodopa (Fig. 3B).

Surprisingly, the addition of 6.4% L-leucine resulted in a decrease in FPF and an increase of the X<sub>50</sub> compared to  $\leq$ 5% L-leucine, particularly for the higher dose weights. A possible explanation, although not investigated, is that this is caused by an observed increased stickiness of the levodopa–leucine mixture at L-leucine amounts over 5%. This has a negative effect on dispersion and inhaler retention.

The inhaler retention was (on average for all formulations with L-leucine) approximately 20% of the metered dose (Fig. 4C). Because it is consistent and fairly well the same for all L-leucine concentrations between 1% and 5%, it is considered acceptable, as it does not significantly influence the consistency of delivered dose.

Although both dispersion and inhaler retention of levodopa improved maximally already after the addition of only 1% L-leucine (Figs. 3 and 4), the co-micronization process with 1% L-leucine was more difficult to control than with 2% L-leucine because of excessive powder adhesion to the fluid energy mill parts. Therefore, we selected 2% L-leucine as optimal and we continued studying this co-micronized formulation in greater detail with both LDA and CIA to obtain more information about its performance.

### 3.5. The effect of pressure drop and metered dose on dispersion of the 2% L-leucine formulation in the Cyclops

#### 3.5.1. Laser diffraction analysis

In order to investigate the effect of the pressure drop (i.e. flow rate) on powder dispersion and to determine the pressure drop minimally needed for good dispersion efficiency, we performed

more LDA measurements. Although it is the kinetic energy of the inhaled airflow that is used to disperse the powder, it is better to take the pressure drop as variable because this is what the patient can generate across the inhaler. The corresponding flow rate through the inhaler depends on its airflow resistance and different inhalers, with different resistances, result in different airflows at the same pressure drop. Obviously, the flow rate (and thus, the available energy for dispersion) increases with increasing (square root of) pressure drop across the same device and this explains the increased FPF and decreased  $X_{50}$  with increasing pressure drop (Fig. 5A and B). Fig. 5A and B also shows a trend towards a lower dispersion efficiency with increasing dose weight for the lower pressure drops (2 and 3 kPa). This effect may be explained by the fact that at lower velocities inside the classifier, powder circulation occurs over a wider cross section of the classifier chamber. At a higher payload, when the particle concentration in the air is much higher, this most likely results in passage of some larger agglomerates by the drag created by the fine aerosol particles leaving the classifier chamber. At 4 and 6 kPa, the FPF <math>5 \mu\text{m}</math> appears to be

independent of the dose weight (Fig. 5A). It is to be noted that pressure drops up to 4 or 6 kPa are easily attainable for the majority of patients across a high resistance inhaler such as the Cyclops. The low flow rate corresponding to 4 kPa (34 L/min) is beneficial as it is likely to limit the oropharyngeal deposition. The inhaler retention presented in Fig. 5C may seem highly variable, but this is largely the result of the use of the gravimetric method for the measuring. Due to the high tare weight of the machined inhaler, results are less reliable and the variation in data is considerably higher than when chemical analysis is performed (see Fig. 6C for comparison). Therefore, it was decided to use chemical analysis for the most promising formulation tested with CIA.

### 3.5.2. Cascade impaction analysis of the most promising levodopa powder

Because levodopa co-micronized with 2% L-leucine showed the best performance during the LDA experiments, we also tested this formulation for doses of 20, 30 and 40 mg with CIA to measure the metered fine particle dose (FPF <math>5 \mu\text{m}</math>) as function of the aerody-

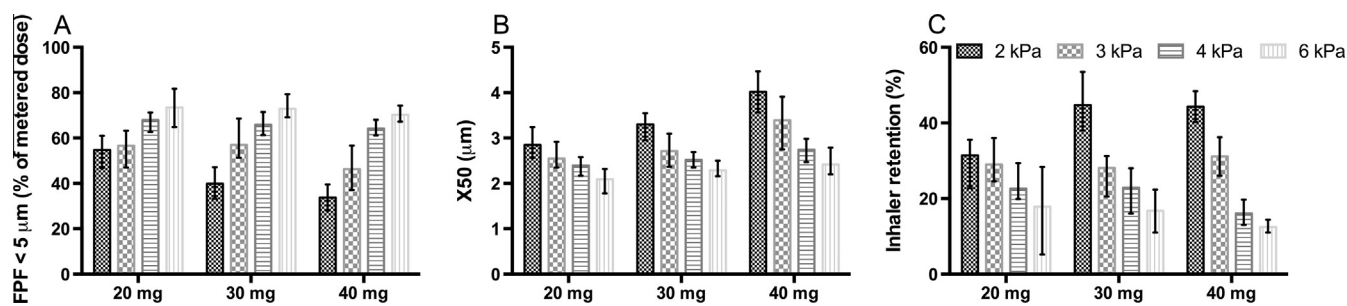


Fig. 5. Effect of pressure drop on (A) FPF <math>5 \mu\text{m}</math> as % of metered dose, (B)

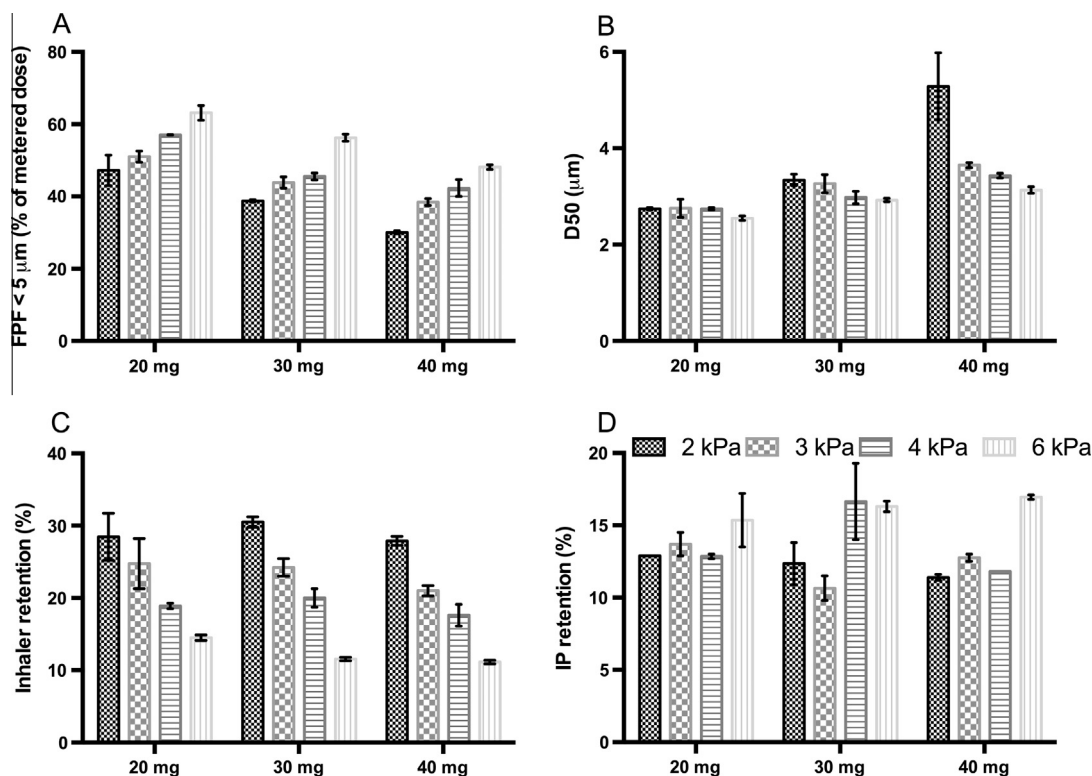


Fig. 6. Effect of pressure drop on (A) FPF <math>5 \mu\text{m}</math> as % of metered dose, (B)

namic diameter (Fig. 6A). For all doses, FPF increased with increasing pressure drop and this is required to obtain a lung deposition that is widely independent of the pressure drop created by the patient. With the use of such inhalers, the shift in the particle deposition towards larger airways (including the oropharynx) at a higher flow rate is (partly) compensated by the higher FPF [15,26]. Additionally, we computed the mass median aerodynamic diameter ( $D_{50}$  or MMAD) of this fine particle fraction (Fig. 6B). The inhaler retentions were measured by chemical analysis (Fig. 6C). We also assessed the losses in the inlet port to the impactor (IP retention) which could be indicative for the losses in the oropharynx as they are related to the velocity and flow pattern with which the aerosol is released from the inhaler's mouthpiece (Fig. 6D). IP retention is comparable at 2, 3 and 4 kPa and only approximately 12.5% of the metered dose. As already mentioned, this may be explained by the high resistance of the Cyclops. Obviously, the inhaler retention decreases with increasing air flow, from about 25–30% at 2 kPa to around 12–15% at 6 kPa and it may be possible to reduce inhaler retention further in future by minor modifications of the inhaler design. Nevertheless, retentions appeared to be fairly consistent for each of the pressure drops and acceptable at 4 and 6 kPa for use in patients.

### 3.5.3. Emission time

Based on the emission time data presented in Table 2 it may be expected that sufficient peripheral deposition from the Cyclops can be achieved in Parkinson's disease patients in an off period with the prepared levodopa formulation containing 2% L-leucine. To reach peripheral parts of the airways, inhalation must be from residual volume, achieved by maximal exhalation, and discharge of the dose from the inhaler should be within the first 1 to 1.5 L of inhaled air [15]. Table 2 compares the times needed to emit 50% and 80% of the delivered dose (Tem 50% and Tem 80% respectively) for different dose weights and different pressure drops. The table also shows the inhaled air volumes in which 80% of the dose is delivered (IV80%). For all doses, at all pressure drops, at least 80% of the delivered dose is emitted from the Cyclops within the first 1 to 1.5 L of inhaled air (L corresponding to IV80%). In a patient characterization study [27] we showed that most Parkinson's disease patients in an off period are able to create a pressure drop of at least 4 kPa for a sufficiently long time to realise this desired inhaled volume of 1.5 L. In this patient characterization study we used a test inhaler with a resistance equal to that of the Cyclops. In addition, these patients were also able to hold their breath for more than 5 s after the inhalation manoeuvre, which is desired for sufficient deposition of the inhaled fine particles by sedimentation in the peripheral airways. Therefore, Parkinson's disease patients in an off period should be able to perform an inhalation manoeuvre that meets the basic requirements for substantial lung deposition of the levodopa formulation containing 2% L-leucine from the Cyclops. This will be tested *in vivo* in a future clinical trial.

**Table 2**

Dose emission times from the Cyclops for levodopa + 2% L-leucine powder as function of the pressure drop. (IV = inhaled volume, IV80%: inhaled volume for 80% dose emission, Tem 50% and Tem 80%: times needed to emit 50% and 80% of the delivered dose.)

$\Delta P$ (kPa)	Dose (mg)	Tem 50% (s)	Tem 80% (s)	IV80% (L)
2	30	1.9	3.5	1.4
3	30	1.5	2.9	1.4
4	20	1.0	1.8	1.0
4	30	1.3	2.5	1.4
4	40	1.4	2.6	1.5
6	30	0.7	1.3	0.9

### 3.5.4. Consistency of delivered dose

The consistency of delivered dose of the co-micronized levodopa formulation with 2% L-leucine was measured at 4 kPa according to the procedures of the European Pharmacopoeia, 8th (21) in 4 L of inhaled air. The mean delivered dose from a metered dose of 30 mg was 22.3 mg (74.2%), with a spread of 21.3 mg to 22.8 mg. This small spread reflects the consistency of inhaler retention presented in Fig. 6 and complies with requirements in the Pharmacopoeia (at least 9 out of 10 delivered doses should be between 75% and 125% of the average, whereas all should be between 65% and 135%). Hence, with this formulation the dose will be consistently delivered to the respiratory tract.

## 4. Conclusions

In this study, a dry powder inhalation formulation of levodopa was developed with a simple micronization technique and minimal use of excipients. The use of laser diffraction technique allowed the fast screening of the dispersion performance of candidate formulations, in the Cyclops, of which only the most promising formulation (with 2% L-leucine) was characterized more extensively with CIA to assess the delivered fine particle dose (FPF <5  $\mu\text{m}$  as per cent of the metered mass). Levodopa co-micronized with only 2% L-leucine and dispersed with the Cyclops high dose dry powder inhaler appears to be a promising candidate for the treatment of patients suffering from Parkinson's disease in an off period. The combination of this particular formulation and inhaler meets the basic *in vitro* requirements regarding emission rate, dispersion efficiency and consistency of delivered dose for satisfactory drug delivery to the lung. This is partly due to the high resistance of the inhaler, which limits the flow rate and, therefore, oropharyngeal deposition. In a different study [27] we showed that the high resistance Cyclops can well be handled and is well accepted by Parkinson's disease patients in an off period. Our results show that the current formulation, when administered with the Cyclops inhaler, is suitable for future clinical trials. These will have to show whether or not the developed levodopa inhalation product can bring the desired therapeutic effect. If so, it may be an attractive alternative to other developments because of its simple and low cost production process, a low health risk due to the restricted use of excipients, a low oropharyngeal deposition, the disposable nature of the inhaler and its ease of handling by the patient.

## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ejpb.2015.10.003>.

## References

- [1] H. Bergman, G. Deuschl, Pathophysiology of Parkinson's disease: from clinical neurology to basic neuroscience and back, *Mov. Disord.* 17 (Suppl. 3) (2002) S28–S40.
- [2] A. Samii, J.G. Nutt, B.R. Ransom, Parkinson's disease, *Lancet* 363 (2004) 1783–1793.
- [3] H.H. Fernandez, A. Vanaganas, P. Odin, A.J. Espay, R.A. Hauser, D.G. Standeert, K. Chatamra, J. Benesh, Y. Pritchett, S.L. Hass, R.A. Lenz, Levodopa-carbidopa intestinal gel in advanced Parkinson's disease open-label study: interim results, *Parkinsonism Relat. Disord.* 19 (2013) 339–345.
- [4] M.A. Cenci, K.E. Ohlin, P. Odin, Current options and future possibilities for the treatment of dyskinesia and motor fluctuations in Parkinson's disease, *CNS Neurol. Disord. Drug Targets* 10 (2011) 670–684.
- [5] D.J. Pedrosa, L. Timmermann, Review: management of Parkinson's disease, *Neuropsychiatr. Dis. Treat.* 9 (2013) 321–340.
- [6] J.J. Chen, C. Obering, A review of intermittent subcutaneous apomorphine injections for the rescue management of motor fluctuations associated with advanced Parkinson's disease, *Clin. Ther.* 27 (2005) 1710–1724.
- [7] I.U. Haq, P.A. Lewitt, H.H. Fernandez, Apomorphine therapy in Parkinson's disease: a review, *Expert Opin. Pharmacother.* 8 (2007) 2799–2809.



- [8] R.T. Bartus, D. Emerich, P. Snodgrass-Belt, K. Fu, H. Salzberg-Brenhouse, D. Lafreniere, L. Novak, E.S. Lo, T. Cooper, A.S. Basile, A pulmonary formulation of L-dopa enhances its effectiveness in a rat model of Parkinson's disease, *J. Pharmacol. Exp. Ther.* 310 (2004) 828–835.
- [9] Civitas Therapeutics, Civitas Therapeutics Announces Positive Phase 2 Clinical Results for CVT-301, an Inhaled L-dopa for Parkinson's disease. CVT-301 shown to treat debilitating OFF Episodes Associated with Parkinson's Disease. (2013). [www.civitastherapeutics.com/news](http://www.civitastherapeutics.com/news). Accessed: June 26th, 2014.
- [10] Civitas Therapeutics, Civitas Therapeutics Initiates Phase 2a Clinical Study of CVT-301, An Inhaled L-dopa for Parkinson's Disease, 2012. <[www.civitastherapeutics.com/news](http://www.civitastherapeutics.com/news)>. (accessed 26.06.14).
- [11] Civitas Therapeutics, Civitas Therapeutics Announces Positive Phase 2b Results for CVT-301, Inhaled Levodopa for the Treatment of Parkinson's Disease, 2014. <[www.civitastherapeutics.com/news](http://www.civitastherapeutics.com/news)> (accessed 26.06.14).
- [12] A.M. Healy, M.I. Amaro, K.J. Paluch, L. Tajber, Dry powders for oral inhalation free of lactose carrier particles, *Adv. Drug Deliv. Rev.* (8) (2014) 32–35.
- [13] M.J. Telko, A.J. Hickey, Dry powder inhaler formulation, *Respir. Care* 50 (2005) 1209–1227.
- [14] D.A. Edwards, J. Hanes, G. Caponetti, J. Hrkach, A. Ben-Jebria, M.L. Eskew, J. Mintzes, D. Deaver, N. Lotan, R. Langer, Large porous particles for pulmonary drug delivery, *Science* 276 (1997) 1868–1871.
- [15] M. Hoppentocht, P. Hagedoorn, H.W. Frijlink, A.H. de Boer, Technological and practical challenges of dry powder inhalers and formulations, *Adv. Drug Deliv. Rev.* (8) (2014) 18–31.
- [16] R. Ho, M. Naderi, J.Y. Heng, D.R. Williams, F. Thielmann, P. Bouza, A.R. Keith, G. Thiele, D.J. Burnett, Effect of milling on particle shape and surface energy heterogeneity of needle-shaped crystals, *Pharm. Res.* 29 (10) (2012) 2806–2816.
- [17] A.H. Chow, H.H. Tong, P. Chattopadhyay, B.Y. Shekunov, Particle engineering for pulmonary drug delivery, *Pharm. Res.* 24 (3) (2007) 411–437.
- [18] H. Steckel, P. Markefka, H. teWierik, R. Kammelar, Effect of milling and sieving on functionality of dry powder inhalation products, *Int. J. Pharm.* 309 (2006) 51–59.
- [19] M. Hoppentocht, O.W. Akkerman, P. Hagedoorn, H.W. Frijlink, A.H. de Boer, The Cyclops for pulmonary delivery of aminoglycosides; a new member of the Twincer family, *Eur. J. Pharm. Biopharm.* (2) (2015) 8–15.
- [20] N. El-Gendy, P. Selvam, P. Soni, C. Berkland, Development of budesonide nanocluster dry powder aerosols: preformulation, *J. Pharm. Sci.* 101 (2012) 3434–3444.
- [21] A.L. Taylor Tavares, G.S. Jefferis, M. Koop, B.C. Hill, T. Hastie, G. Heit, H.M. Bronte-Stewart, Quantitative measurements of alternating finger tapping in Parkinson's disease correlate with UPDRS motor disability and reveal the improvement in fine motor control from medication and deep brain stimulation, *Mov. Disord.* 20 (2005) 1286–1298.
- [22] A.H. de Boer, D. Gjaltema, P. Hagedoorn, M. Schaller, W. Witt, H.W. Frijlink, Design and application of a new modular adapter for laser diffraction characterization of inhalation aerosols, *Int. J. Pharm.* 249 (2002) 233–245.
- [23] F. Grasmeyer, P. Hagedoorn, H.W. Frijlink, A.H. de Boer, Characterisation of high dose aerosols from dry powder inhalers, *Int. J. Pharm.* 437 (2012) 242–249.
- [24] European Pharmacopoeia, eight ed., Supplement 8.2 ed., Directorate for the Quality of Medicines & HealthCare of the Council of Europe (EDQM), 2013.
- [25] E.J. Pappert, C. Buhrfiend, J.W. Lipton, P.M. Carvey, G.T. Stebbins, C.G. Goetz, Levodopa stability in solution: time course environmental effects and practical recommendations for clinical use, *Mov. Disord.* 11 (1) (1996) 24–26.
- [26] P. Demoly, P. Hagedoorn, A.H. de Boer, H.W. Frijlink, The clinical relevance of dry powder inhaler performance for drug delivery, *Respir. Med.* (8) (2014) 1195–1203.
- [27] M. Luinstra, A.W. Rutgers, H. Dijkstra, J. Grasmeyer, P. Hagedoorn, J.M. Vogelzang, H.W. Frijlink, A.H. de Boer, Can patients with Parkinson's disease use dry powder inhalers during off periods?, *PLoS One* 10 (7) (2015)