

**Universidade de Lisboa  
Faculdade de Farmácia**



# **Cannabidiol and Hydroxypropyl- $\beta$ -cyclodextrin Inhalable Powder: A Breakthrough in Spray Drying Technology**

**Bernardo Filipe Santos Gomes**

Trabalho de campo orientado pela Professora Doutora Anna Lechanteur,  
Professora Associada da Universidade de Liège e coorientado pela Professora  
Doutora Helena Maria Cabral Marques, Professora Catedrática da Faculdade de  
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**Mestrado Integrado em Ciências Farmacêuticas**

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## Resumo

Ao longo dos anos, a investigação sobre a administração pulmonar de fármacos avançou significativamente. Foram desenvolvidas estratégias inovadoras para enfrentar os desafios inerentes a esta via de administração, com o objetivo de aumentar a fração respirável de fármaco, normalizar os atributos dos produtos inaláveis e obter consistência na administração da dose.

Recentemente, surgiram diversos dispositivos para administração pulmonar, com destaque para os inaladores de pó seco devido à sua elevada estabilidade, ausência de contaminantes do ar e uso simplificado, ativado pela inspiração do utilizador.

A relação desta tecnologia com as abordagens terapêuticas que utilizam o canabidiol (CBD) revela uma via promissora para o tratamento de um vasto espectro de doenças localizadas e sistémicas. Embora as indicações terapêuticas definitivas para o CBD ainda não tenham sido definidas, um grande número de evidências sugere a sua eficácia em múltiplas vias fisiológicas, comprovando o seu potencial para diversas aplicações terapêuticas.

Neste estudo, foram desenvolvidos pós para inalação com CBD utilizando a tecnologia de *spray drying*. Os promotores de solubilidade, nomeadamente a hidroxipropil- $\beta$ -ciclodextrina e o etanol, foram integrados nas formulações por forma a aumentar a quantidade de CBD nos pós atomizados.

As partículas atomizadas foram submetidas a uma série de análises. As imagens SEM revelaram formas colapsadas em todas as formulações, com um número e profundidade consistentes de depressões. A análise do tamanho das partículas demonstrou baixa polidispersibilidade (aproximadamente 3,3) e tamanhos dentro do intervalo adequado para a deposição pulmonar profunda, variando de 2,01 a 2,14  $\mu\text{m}$ .

A concentração mais elevada de CBD (9,5 mg/g) foi observada no pó de HP- $\beta$ -CD/CBD com 20% de conteúdo sólido. De facto, um teor de sólidos mais elevado nas soluções de alimentação está correlacionado com uma maior quantidade de CBD no pó resultante. Em oposição, o etanol provou estar associado a uma concentração reduzida de CBD nos pós produzidos, apresentando um impacto negativo na formulação.

Em suma, os pós para inalação produzidos induziram elevadas frações de partículas finas (> 56,98%), provavelmente atribuíveis à sua superfície colapsada. Além disso, a

tecnologia de secagem por aspersão facilitou a modulação precisa da morfologia dos pós de CBD sem comprometer a sua estabilidade.

**Palavras-chave:** Canabidiol; *Spray drying*; Inalação; Inalador de pó seco; Hidroxipropil- $\beta$ -ciclodextrina

# Abstract

Over the years, our understanding and investigation into pulmonary drug delivery have advanced significantly. Innovative strategies have been developed to address inherent challenges associated with this delivery route, aiming to enhance drug respirable fraction, standardizing the attributes of inhalable products and achieve consistency in dose delivery.

Recently, various devices for lung administration have emerged, showcasing significant promise. Notably, the dry powder inhaler stands out as the most promising option, primarily due to its considerable advantages, including high formulation stability, absence of air contaminants, and simplified usage procedure, as being triggered by the user's inspiration.

Relating this technology to therapeutic approaches employing cannabidiol (CBD) reveals a promising avenue for treating a wide spectrum of both localized and systemic diseases. While definitive therapeutic indications for CBD are yet to be precisely defined, a wealth of evidence suggests its efficacy in multiple physiological pathways, underscoring its potential for diverse therapeutic applications.

In this study, cannabidiol-engineered inhalation powders were developed using spray drying technology. Solubility enhancers, namely HP- $\beta$ -CD and ethanol were integrated in the formulations to address higher CBD quantity in atomized powders.

Atomized particles were submitted to a range of analysis. SEM images revealed deflated shapes in all formulations, with a consistent number and depth of dimples. Particle size analysis demonstrated low polydispersity (approximately 3.3) and sizes within the suitable range for deep lung deposition, ranging from 2.01 to 2.14  $\mu\text{m}$ .

The highest concentration of CBD (9.5 mg/g) was observed in HP- $\beta$ -CD/CBD powder with 20% solid content. Indeed, higher solid content in the feeding solutions correlated with a greater quantity of CBD in the resulting powder. Conversely, ethanol linked to a reduced concentration of CBD in the powders, displaying a detrimental impact on the formulation.

Overall, the engineered inhalation powders induced high fine particle fractions (> 56.98%), likely attributable to their deflated surface. Moreover, spray drying technology facilitated precise modulation of the morphology of CBD powders without compromising formulation stability.

**Keywords:** Cannabidiol; Spray Drying; Inhalation; Dry powder inhaler; Hydroxypropyl-beta-cyclodextrin

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5 years. 5 years of change. The degree not only gives us tools to learn about medicines, but it also gives us strength to overcome hard times, bond with others and grow as a person. For that, all the people we meet all the way turn to be part of our lives. Friends are the family we have left behind and the one that we miss all days. Professors are the guides we follow to walk a step closer of reaching our dreams.

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# Abbreviations

APSD- Aerodynamic particle size distribution

C- Corrugation

CBD- Cannabidiol

$C_c$ - Cunningham slip correction

COPD- Chronic Obstructive Pulmonary Disease

D- Deflation

d- Physical diameter

$D_a$ - Aerodynamic diameter

Dd- Depth of dimples

DPI- Dry powder inhaler

ED- Emitted dose

EF- Emitted fraction

EtOH- Ethanol

FPD- Fine particle dose

FPF- Fine particle fraction

GDS- Geometric standard deviation

HPLC- High performance liquid chromatography

HP- $\beta$ -CD- Hydroxypropyl-beta-cyclodextrin

IC- Carr compressibility index

ICH- International Council for Harmonization

IH- Hausner index

MDI- Metered-dose inhaler

MMAD- Median mass aerodynamic diameter

Nd- Number of dimples

NGI- Next generation impactor  
PTFE- Polytetrafluoroethylene  
RD- Recovery dose  
RF- Recovery fraction  
SD- Standard deviation  
SEM- Scanning electron microscopy  
SMI- Soft-mist inhaler  
T<sub>g</sub> – Glass transition temperature  
TGA- Thermogravimetric analysis  
UV- Ultraviolet  
Δ<sup>9</sup>- THC- delta-9-tetrahydrocannabinol  
ρ<sub>p</sub>- Particle density

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# 1 Introduction

## 1.1 Pulmonary drug delivery as an advanced therapy

Pulmonary drug delivery has emerged as a focal point of interest in the realm of innovative, effective, and safe approaches for treating both localized and systemic diseases (1).

Currently, inhalation stands as the preferred route of administration for delivering drugs aimed at treating respiratory disorders such as asthma and chronic obstructive pulmonary disease (COPD) (2). However, new formulations have expanded its utility to address chronic conditions like diabetes, cystic fibrosis, fungal and bacterial infections, and pulmonary arterial hypertension (3). This preference for inhalation is attributed to several advantages, including bypassing first-pass metabolism, enhancing bioavailability, achieving faster absorption and onset of action, as well as being a non-invasive method that typically requires lower doses of active substances (4).

Despite the potential benefits, the efficacy of inhaled therapy can be hindered by various factors, including biological barriers and pharmaceutical aspects of the medication. Key pharmaceutical factors include the performance of aerosolization as well as the size and shape of particles (5). The ideal aerodynamic diameter falls within the range of 1 to 5  $\mu\text{m}$ , enabling penetration and sedimentation within the trachea-bronchial region (6).

The human pulmonary airways are functionally divided into two distinct zones: the conducting channels and the respiratory area (1). These airways are lined with respiratory epithelium and mucosa, enabling drug delivery and absorption throughout the respiratory tract. However, the deepest regions offer the best opportunities for dissolution and absorption of inhalable particles (7).

In the modern era, drug aerosolization for lung administration can be achieved through four primary systems: nebulizers, metered-dose inhalers (MDIs), soft-mist inhalers (SMIs), and dry powder inhalers (DPIs). Each of these systems serves specific purposes and offers unique advantages in delivering pharmaceutical agents to the respiratory system (8).

## 1.2 The science behind dry powder inhalers

Dry powder inhalers (DPIs) are pharmaceutical devices designed for the inhalation of bioactive substances in solid form, facilitating their delivery to the lungs. Typically, these devices consist of three integral and distinct functional components: the powdered drug formulation, the drug dose measuring system, and a physical mechanism that enables the dispersion of the powdered formulation (9).

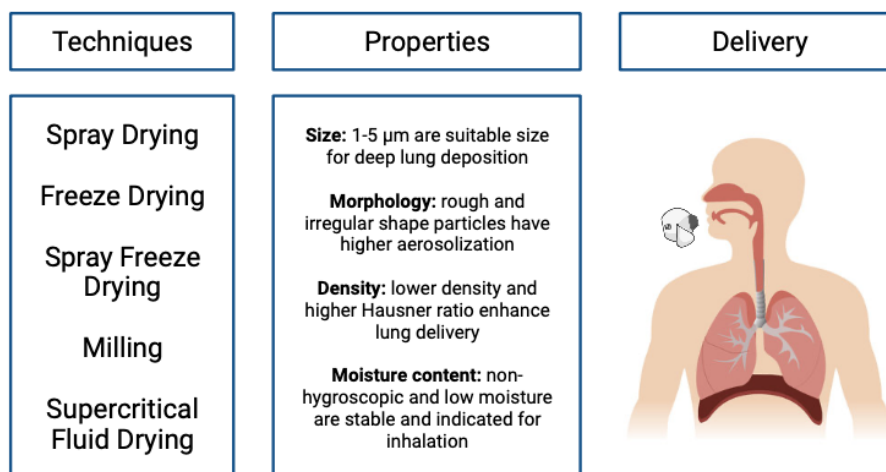
In comparison with other inhalable devices, DPIs demonstrate several advantages as improved product stability since it has less water content compared to liquid formulations, more eco-friendly once no propellant is required, ease of use since there's no need of coordination between the inhalation of patients and actuation of device and capability of high-dose delivery (1).

The underlying mechanism behind this drug delivery strategy involves the generation of airflow within the device. This airflow leads to the disruption of larger particles, a phenomenon known as deagglomeration. As a result of deagglomeration, fine particles are produced, making them more readily transportable to the pulmonary tract due to negative pressure. At this point, drug particles become separated from the carrier and are directed to various regions within the lungs. Consequently, the extent of deposition within the lungs is influenced by three primary factors: the patient's inspiratory effort, the characteristics of the device and the properties of loaded powder itself (10).

When it comes to formulating DPI, there are commonly two approaches that can be pursued. The majority of DPIs incorporate a carrier, such as lactose, mannitol, or trehalose. These carriers serve multiple purposes within the formulation. Primarily, they prevent the aggregation of powder particles and enhance the flowability of the mixture. Additionally, carriers add bulk to the product, facilitating metering, dispensing, and handling of the formulation. Given that the carrier constitutes the largest portion of all compounds in the formulation, its properties play a significant role in influencing the aerosol performance of the DPI (9). Other way of producing an inhalable powder is as loose clusters comprising engineered powder, where carrier is not added (11).

Inhalable dry powder formulations intended for use in DPIs can be manufactured using various techniques. Among the commonly employed methods, demonstrated in Figure 1 are milling and spray drying. Less frequently used but still relevant techniques include spray freeze drying and supercritical fluid drying. Furthermore, emerging processes like thin film

freezing and hot melt extrusion hold promise as potential methods for producing inhalable dry powder formulations (12).



**Figure 1- Illustrative scheme of main techniques and powder's properties in the field of inhalable powder's production. [Adapted from (12)]**

### 1.3 Particle engineering as a precision tailoring strategy

The development of an effective dry powder manufacturing process presents numerous challenges, essentially stemming from the inadequate aerosolization of the powder due to unfavourable characteristics inherent to micronized materials (13). At the microscopic scale, these micron-sized particles possess a significant amount of surface free energy, resulting in pronounced cohesiveness. This inherent property leads to suboptimal flowability and a strong tendency for particles to aggregate, thereby compromising their dispersibility. Consequently, particle-level engineering emerges as a strategic approach to mitigate these challenges, harmonizing the forces within the dry powder bulk. This optimization ensures stability throughout processing and storage and facilitates the generation of fine particles during the inhalation process (14).

Particle engineering assumes paramount importance by enabling the tailored modification of various physicochemical attributes, including size, morphology, and water content, among others. Moreover, it contributes to the establishment of reproducible product characteristics through precise control of equipment parameters (15).

Aerosolized engineered particles conferred several advantages such as better flowability, high homogeneity and high fine particle fraction over non-engineered particles (16).

The size of aerosolized particles directly impacts their penetration into the airways. Larger particles ( $> 5 \mu\text{m}$ ) tend to settle in the upper and larger airways, while smaller particles ( $< 1 \mu\text{m}$ ) are inhaled and exhaled. Particles falling within the 1 to 5  $\mu\text{m}$  range are well-suited to reach the alveolar region, enhancing lung drug delivery (17).

Most inhalable particles present non-spherical shape, which leads to the employment of aerodynamic diameter ( $D_a$ ) term as the diameter of a unit-density sphere with the same settling velocity as the particle in question (18). It allows the evaluation of particle motion in a fluid, taking in account the shape, surface structure and density of the particle. It can be calculated multiplying physical diameter ( $d$ ) by square root of multiplication of particle density ( $\rho_p$ ) and Cunningham slip correction ( $C_c$ ) (19).

The density of fine particles plays a pivotal role in their effectiveness for drug delivery. Lower density and higher Hausner values are associated with improved aerosolization properties. Porous particles, characterized by a density below  $0.5 \text{ g/cm}^3$  and a substantial surface area exceeding  $50 \text{ m}^2/\text{g}$ , contribute to a smaller aerodynamic diameter. This unique feature limits inter-particulate cohesive forces, reducing particle agglomeration and ultimately enhancing lung delivery (15).

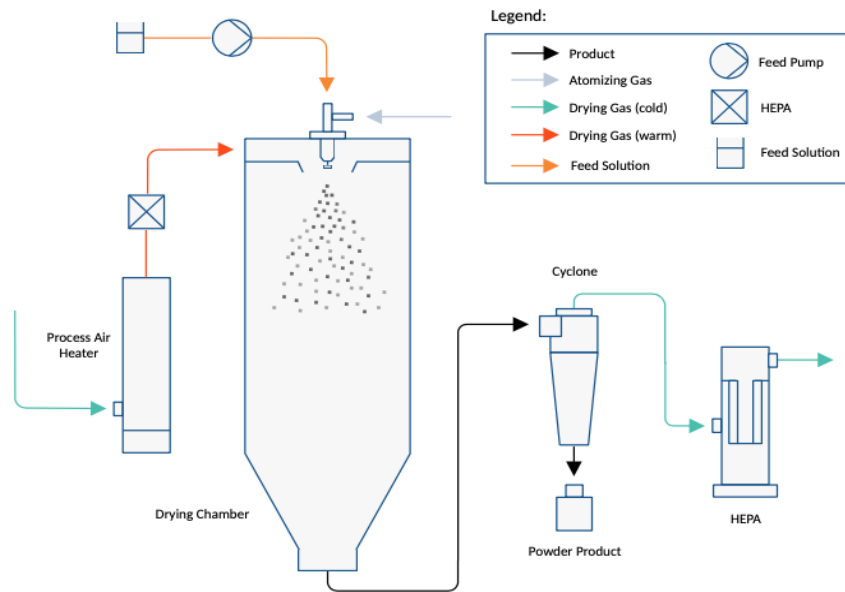
The shape or morphology of particles has a direct influence on the aerodynamic diameter, affecting how particles agglomerate and their specific surface area as well as friction (15). Powders composed of smooth-surfaced particles do not exhibit significant deposition in the deeper parts of the lungs. Conversely, particles with a corrugated or deflated structure show higher efficiency in this regard. Nevertheless, extreme morphologies characterized by numerous dimples or deep indentations result in poor deposition within the deep pulmonary regions (20).

#### **1.4 The technology of spray drying**

Concerning to respiratory drug delivery, spray drying technology has emerged as the predominant technique for powder manufacturing (21). This process effectively converts a liquid feedstock (comprising solutions, suspensions, or emulsions) into a dry powder. Spray

drying is widely recognized as an efficient tool for tailoring the physicochemical characteristics of a material, thereby enhancing its aerosol performance (22).

A spray dryer, as presented in Figure 2, is constituted of a feed pump, atomizer, air heater, air dispenser, drying chamber, collection chamber, cyclone, and waste filter. The operational procedure comprises three primary phases (23).



**Figure 2- Schematic representation of the components of a spray dryer. [Adapted from (24)]**

The initial phase involves atomization, employing a nozzle to disintegrate the feeding liquid into fine droplets, which are then dispersed in a flowing air stream for drying through the action of compressed gas (25). This results in the enormous increase of surface area of the bulk fluid, causing a reduction of the internal resistance to moisture transfer from droplet to the surrounding medium (26).

Subsequently, the drying operation takes place within a dedicated chamber, where filtered and heated air contacts with the atomized droplets. The solvent present in the dispersed droplets undergoes vaporization, resulting in the formation of solid particles (27). Simultaneous heat and mass transfer occur, where heat is transferred from air to droplet by convection. Vapor is transported from the droplet to air by convection through the droplet boundary layer (28).

Lastly, the separation step occurs in a cyclone, where spray-dried particles are isolated from the drying gas and collected into a designated container. Using this separator, particles are segregated from the continuous gas stream, relying on differences in density. This separation is achieved by subjecting the particles to an accelerating flow field within a rotating vortex generated within the cyclone (25).

The atomization method under consideration can be described as rapid, continuous, highly reproducible, single-step, and readily scalable without requiring significant modifications (29). Regarding to powder production, spray drying offers distinct advantages when compared to alternative technologies. These advantages include the capacity to finely control particle size, shape, porosity, and density, which are contingent on the manipulation of various process parameters. Furthermore, it allows the utilization of different forms (solution, emulsion, and suspension) as feeding solutions in a unified, single-step procedure, yielding highly dispersible and spherical particles characterized by a narrow size distribution. Additionally, this method is widely regarded for its simplicity, cost-effectiveness, and ease of scalability (30).

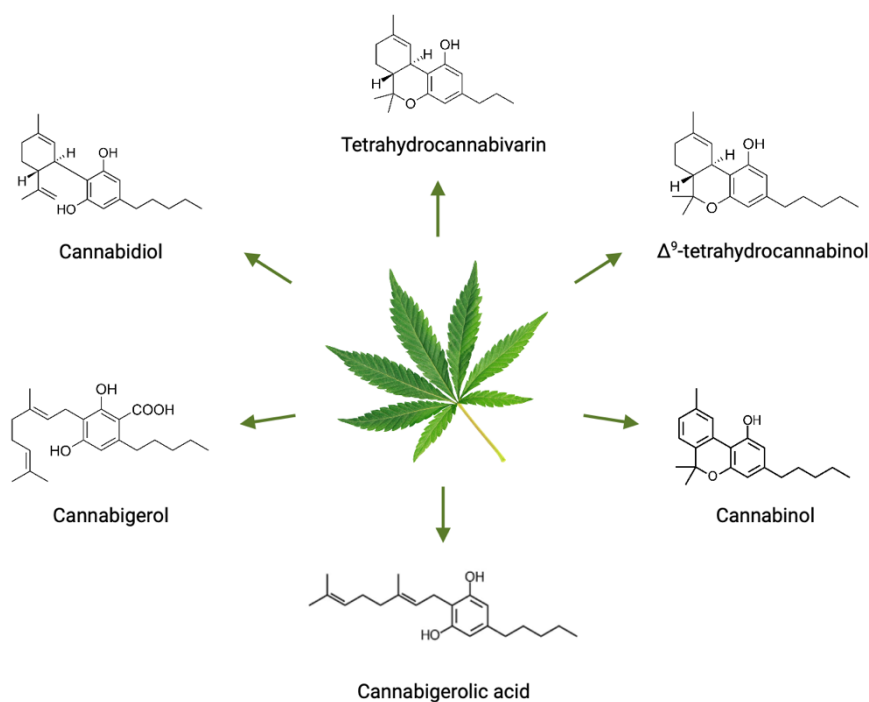
At each stage of the process, the equipment's parameters assume a critical role in shaping the attributes of the final product, such as particle size, density, and morphology. These characteristics are subject to influence by an array of process parameters, including the atomization method, atomizer configuration, feed solids concentration, feed physical properties, and inlet and outlet temperatures (26).

In terms of the physical attributes of the feeding liquid, it is essential to consider factors such as viscosity, surface tension, and solid content, as these play a pivotal role in defining the characteristics of the spray-dried particles (31). To ensure the protection of bioactive substances during the atomization process and improve storage stability, specific excipients, particularly disaccharides, have been deliberately employed. Scientific investigations suggest that this safeguarding is achievable for two main reasons: the formation of an amorphous glass during the process, resulting in minimal molecular alterations, and the establishment of hydrogen bonding between the sensitive component and the excipient (32).

## **1.5 Cannabidiol's broad-spectrum drug properties**

The herbaceous plant *Cannabis sativa*, as classified by Linneo in 1753, has long been the subject of exploration due to its medicinal potential. It has demonstrated various therapeutic

properties, serving as an analgesic, anticonvulsant, anti-asthmatic, antimalarial, and anti-rheumatic agent (33). This diverse array of applications is attributable to the plant's rich composition of secondary metabolites, encompassing terpenoids, flavonoids, alkaloids, lignans, and around 113 compounds with a complex biogenic origin, collectively referred to as phytocannabinoids, some demonstrated in Figure 3 (34). Among these phytocannabinoids, the two principal constituents are  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC) and cannabidiol (CBD), which can be detected in cannabis extracts at levels of 17.3% and 9.6%, respectively (35).



**Figure 3- Representative image of a *Cannabis sativa* plant and a selection of its extracted compounds. [Adapted from (36)]**

The biological effects of CBD and  $\Delta^9$ -THC on the human body are notably distinct.  $\Delta^9$ -THC is primarily responsible for the behavioral effects associated with the consumption of *Cannabis sativa* preparations. These effects include paranoia, memory impairment, an elevated risk of psychotic illness, and the potential for developing cannabis dependency (37).

Conversely, CBD has demonstrated a wide range of effects across various conditions, such as anxiety, inflammation, neuropathic pain, and epilepsy. Current understanding suggests that CBD interacts with different molecular targets, implying that its effects are likely the result of multiple mechanisms of action (38).

CBD's multifaceted properties have unveiled significant therapeutic potential without the associated psychotropic adverse effects. Nevertheless, our comprehension of the neuro-molecular mechanisms underlying CBD's actions remains limited, and its complex pharmacology has yet to be fully elucidated. This knowledge gap leaves room for further exploration and the discovery of additional therapeutic applications (39).

CBD exhibits a low oral bioavailability, with only 6% of the administered dose typically reaching the bloodstream. In contrast, when administered via the pulmonary route, it demonstrates a more favorable pharmacokinetic profile. This results in a significant increase in bioavailability to 31%, along with a considerably faster absorption profile, occurring within 5 minutes as opposed to the 2 hours required for oral administration. Additionally, the peak concentration in the bloodstream is 24-fold higher when using the pulmonary route. By allowing for the administration of lower CBD doses, the risk of adverse effects can be minimized, making these inhalable medicines safer and more tolerable for patients (40).

CBD possesses distinct physicochemical characteristics, which present formulation challenges. It exhibits a remarkably low water solubility, measuring only 12.6 mg/L, and boasts high lipophilicity with a logP of 6.3 (41). Furthermore, the CBD molecule initiates degradation at temperatures exceeding 160 °C (42).

To tackle these solubility issues, strategies such as employing the amorphous form of CBD, despite its thermal instability, have been explored. This amorphous state can lead to recrystallization concerns, which can be effectively reduced through the incorporation of stabilizing polymers. Given these compelling considerations, the incorporation of hydroxypropyl-beta-cyclodextrin (HP- $\beta$ -CD) emerges as a promising approach to enhance solubility and safeguard against temperature-induced degradation (43).

## 2 Objectives

The primary objective of this study was to formulate a cannabidiol-loaded powder designed for lung administration. Our investigation primarily focused on complexation with hydroxypropyl-beta-cyclodextrin (HP- $\beta$ -CD), atomization through spray drying technology and comprehensive characterization of the cannabidiol powders. Additionally, our objective was to understand the impact of both quantitative and qualitative variations in the formulation on the physicochemical properties of the collected particles.

Furthermore, it was aimed to evaluate the viability of employing the spray drying technique in the manufacturing process of inhalable powders, taking into careful consideration the specific requirements of this route of administration. Lastly, we sought to assess the aerodynamic particle distribution profile and correlate it with the modifications applied to the formulation.

## 3 Materials and methods

### 3.1 Materials

Hydroxypropyl- $\beta$ -cyclodextrin (HP- $\beta$ -CD) (Kleptose HPB- molar substitution= 0.63) was provided by Roquette (Lestrem, France). Cannabidiol (CBD) was obtained from THCPharm GmbH (Frankfurt, Germany). Ethanol absolute was supplied by Thermo Fisher Scientific (Loughborough, England). Ultrapure water was produced by a Rephile PURIST PRO water purification system.

### 3.2 Preparation of the solution before atomization

Three distinct solutions were prepared, each containing both CBD and HP- $\beta$ -CD. In two of these solutions, the excipients (characterized physiochemically in Table 1) were dispersed in deionized water, with one containing 15% solid content and the other 20% solid content. The third solution employed an aqueous medium with 10% (v/v) ethanol and 15% solid content.

The volume of each solution was standardized at 10 mL. The ethanol-free solution with 15% solid content and the ethanol-containing solution were made in triplicate, while the 20% solid content solution was prepared only once. They were all made following the same procedure:

CBD (1.76%) and HP- $\beta$ -CD (98.24%) were accurately weighed and dispersed in the specified solvents. The resulting samples underwent 5 minutes of ultrasounds waves, followed by placement in a thermostated bath set to 37 °C with continuous agitation for a duration of 24 hours.

Any excess CBD particles that remained unsolubilized were eliminated using a syringe membrane filter made of PTFE with a pore size of 0.45  $\mu$ m. 500  $\mu$ L of filtered solution were transferred to Eppendorf tubes and set aside for subsequent analysis.

**Table 1. Properties of the excipients included in stock formulations.**

Substance	Function	Molecular weight (g/mol)	Tg (°C)	Flash point (°C)	Water Solubility
HP-β-CD	Carrier and solubilizer	1395.30	220.40 (44)	311.8	> 450 g/L at 20 °C
Ethanol	Co-solvent and solubilizer	46.07	-176.15 (45)	13	Miscible

HP-β-CD – Hydroxypropyl-beta-cyclodextrin; Tg – Glass transition temperature. Data collected from excipient's data sheet, except Tg from literature.

### 3.3 Spray drying

The equipment used in this operation was a Procept 4 M8-Trix Formatrix spray dryer (Procept, Zelzate, Belgium) with a bi-fluid nozzle. The parameters of the process are shown in Table 2 (20,46). The process yield was calculated for each powder obtained with the following formula:

$$\text{Process yield (\%)} = \frac{\text{Mass of obtained spray dried powder (g)}}{\text{Mass of total dissolved powder before spray drying (g)}} \times 100 \quad (1)$$

**Table 2. Parameters of spray drying process.**

Parameter	Specification
Solid content	15% or 20% (w/w)
Cyclone gas pressure	0.4 bar
Inlet gas flow	0.4 m <sup>3</sup> /min
Inlet temperature	160 °C
Pump speed	100 rpm
Flow rate	3.85 g/min
Nozzle gas pressure	3 bar
Nozzle diameter	0.4 mm

### **3.4 CBD quantification**

High performance liquid chromatography (HPLC) was used to quantify CBD using an HPLC Agilent serie1100 with OpenLab CDS LC ChemStation version C.01.05 as the software, UV detector operating at 240 nm and with a Zorbax® C18 300 SB 4.6\*150 mm analytical column filled 3.5 µm C18 (X Bridge BEH C18). The mobile phase was composed of a mixture of water and acetonitrile [38/62% (v/v)]. The flow rate was set at 1.0 ml/ min and the column was kept at 30 °C. 20 µL of the samples were injected at room temperature, the chromatographic run time was set to 10 min.

The process was fully validated based on the total error as a decision criterion. The acceptance limits were set at 10%. All validation results were computed using the Enoval software V3.0 (Arlenda, Liege, Belgium).

### **3.5 Particle size distribution measurement**

To assess the size characteristics of the generated powder particles, a laser diffraction particle size analyzer, specifically the Mastersizer 2000, coupled to Scirrocco powder feeder (Malvern, UK), was employed. A dispersion pressure of 4 bars was used, with a measurement duration of 10 seconds. Approximately 150 mg of powder was utilized for each sample to achieve the desired obscuration within the range of 0.5% to 5%.

### **3.6 Scanning electron microscopy**

The particulate morphology was observed by scanning electron microscopy (SEM) using either a Philips XL30 ESEM or a FEI Quanta 600 after metallization with Au (~50 nm). Representative micrographs were captured, and four particles were sampled for each powder to measure their diameter and characterize their shape. For each powder, the average number of dimples was manually counted (the visible dimples can be assumed to be half the total number of a particle), and dimple depth (normalized to the diameter  $d$  of the particle) was estimated.

### 3.7 Thermogravimetric analysis

The quantification of residual moisture in the obtained powders was performed using a Thermogravimetric analysis (TGA 2- Mettler Toledo, Switzerland). Powder samples ranging from 6 to 12 mg were placed into a platinum sample pan. The method used started at 25 °C and reached 200 °C at a rate of 10 °C/min.

### 3.8 Bulk and tapped density

Based on previous experiments and research, it was already established that the Hausner ratio of powders containing the same substances would be higher than desired. Consequently, this analysis was only conducted on a single powder.

Bulk and tapped density were performed according to European Pharmacopoeia Procedure 2.9.34 (<https://www.edqm.eu/en/european-pharmacopoeia-ph.-eur.-11th-edition>). Because of the limited sample quantity, a 10 mL graduated cylinder was used to measure a certain volume of powder. The bulk volume used for the calculation of the bulk density was directly read from the cylinder.

$$\text{Bulk density (g/cm}^3\text{)} = \frac{\text{Wheight of powder (g)}}{\text{Bulk volume of powder (cm}^3\text{)}} \quad (2)$$

The tapped density was determined by mechanically tapping a graduated cylinder containing the powder sample. The value of the tapped volume was read after 10 and 500 taps at a tapping height of 3 mm.

$$\text{Tapped density (g/cm}^3\text{)} = \frac{\text{Wheight of powder (g)}}{\text{Tapped volume of powder (cm}^3\text{)}} \quad (3)$$

The Hausner (IH) and Carr compressibility indexes (IC%) are used to express flowability of powders. Those were calculated based on tapped and bulk densities.

$$IH = \frac{\text{Tapped density}}{\text{Bulk density}} \quad (4)$$

$$IC\% = \frac{\text{Tapped density} - \text{bulk density}}{\text{Tapped density}} \quad (5)$$

### 3.9 Homogeneity and drug recovery

For each formulation, homogeneity was assessed by analyzing five spray dried powder samples from every batch. Each sample was dissolved in a fixed volume of a 62:38 (v/v) of acetonitrile and water solution. After a brief sonication, the resulting solutions underwent filtration using a 0.45 µm PTFE membrane filter and were subsequently subjected to HPLC analysis (as detailed in section 3.3). After each production run of powder, homogeneity was evaluated, and CBD recovery was quantified using the following formula.

$$\text{Recovery (\%)} = \frac{\text{Total mass of CBD in spray dried powder}}{\text{Total mass of CBD in solution (before SD)}} \times 100 \quad (6)$$

### 3.10 *In vitro* lung deposition evaluation

Aerodynamic particle size distribution was characterized for each batch of produced powder through *in vitro* analysis conducted using a Next Generation Impactor (NGI) equipment. The dry powder inhaler (DPI) used was the Aerolizer® device, which was connected to a mouthpiece that was attached to an induction port and a preseparator. This cascade impactor comprises eight stages, each demarcated by a pore diameter that encompasses a particle size range spanning from 0.206 µm to 12.8 µm. The pressure and flow rate parameters were controlled through a pump system interlinked with the equipment.

For each batch, twelve size 3 capsules were filled with a 30 mg quantity of spray dried powder and subjected to a one-hour deionization process. Subsequently, one capsule at a time was punctured using the inhaler and exposed to a controlled airflow of 100 ml/min for precisely 2.4 seconds. Following the introduction of all twelve capsules, the powder deposited at each of the defined stages was recovered utilizing a solution composed of 62% acetonitrile and 38% water, and subsequently subjected to HPLC analysis.

The resulting mass located in the throat and stages 1 to 8 was designated as the recovery dose (RD). Therefore, the recovery fraction (%) was calculated by considering the total dose encompassed within the twelve capsules. The fine particle dose (FPD) was specifically quantified as the aggregate mass of particles with dimensions of up to 5 µm. Furthermore, the fine particle fraction (FPF) was derived by dividing the FPD by RD, expressed as a percentage. The mass median aerodynamic diameter (MMAD) was meticulously calculated from the cumulative curve of aerosol mass distribution, representing the diameter at which

50% of the total mass can pass. Finally, the geometric standard deviation (GSD) was computed employing the relation  $GSD = D_{84}/D_{50}$ .

## 4 Results

**Table 3. Characteristics of spray dried powders.**

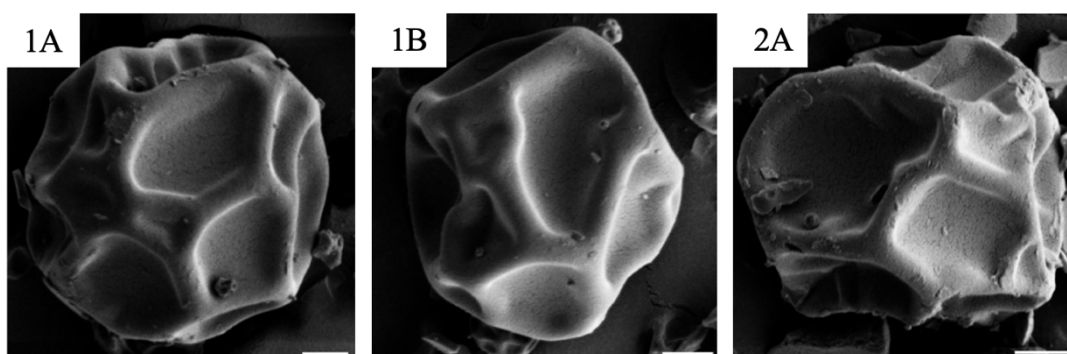
Characteristics	HP-β-CD/CBD		HP-β-CD/CBD/EtOH
	15% solid content	20% solid content	15% solid content
Column Out Temperature (°C)	85.14 ± 1.06	85.50	83.65 ± 2.51
Process yield (%)	55.57 ± 8.94	68.42	68.83 ± 3.57
Particle size d50 (µm)	2.05 ± 0.28	2.14	2.01 ± 0.10
Span (-)	3.33 ± 0.25	3.25	3.36 ± 0.22
Water content (%)	3.52 ± 0.38	4.11	5.22 ± 0.17

CBD – Cannabidiol; HP-β-CD – Hydroxypropyl-β-cyclodextrin; EtOH – Ethanol. Values are expressed as mean ± SD. n = 3 (except in powder of 20% HP-β-CD/CBD, which was one sample)

**Table 4. Spray dried particles aspects.**

Particle properties	HP-β-CD/CBD		HP-β-CD/CBD/EtOH
	15% solid content	20% solid content	15% solid content
Nd	21.50 ± 3.49	16.50 ± 2.14	19.00 ± 4.49
Dd	0.14 ± 0.02	0.15 ± 0.03	0.16 ± 0.06
D	0.64 ± 0.09	0.80 ± 0.06	0.68 ± 0.10
C	0.36 ± 0.11	0.40 ± 0.07	0.38 ± 0.04

CBD – Cannabidiol; HP-β-CD – Hydroxypropyl-β-cyclodextrin; EtOH – Ethanol; Nd – Number of dimples; Dd – Depth of dimples; D – Deflation; C – Corrugation. Values are expressed as mean ± SD. n = 4



**Figure 4- Scanning electron microscopy (SEM) images of spray dried particles from feeding solutions containing 15% of HP-β-CD/CBD (1A), 20% of HP-β-CD/CBD (1B) and 15% of HP-β-CD/CBD/EtOH (2A). Scale bar = 1 μm.**

**Table 5. CBD quantification during powder's production.**

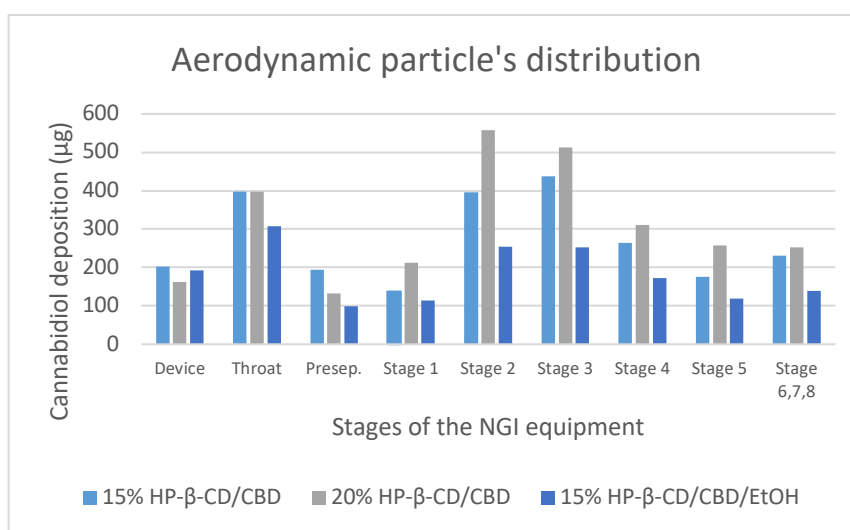
CBD quantification	HP-β-CD/CBD		HP-β-CD/CBD/EtOH
	15% solid content	20% solid content	15% solid content
[CBD] in solution (g/L)	1.07 ± 0.08	2.02	0.74 ± 0.25
[CBD] in SD powder (mg/g)	7.66 ± 0.40	9.50	4.83 ± 0.22
Drug recovery (%)	118.36 ± 2.85	100.01	103.27 ± 3.08

CBD – Cannabidiol; HP-β-CD – Hydroxypropyl-beta-cyclodextrin; EtOH – Ethanol; SD – Spray dried. Values are expressed as mean ± SD. n = 3 (except in powder of 20% HP-β-CD/CBD, which was one sample)

**Table 6. Aerodynamic behavior of spray dried powders.**

Aerodynamic properties	HP-β-CD/CBD		HP-β-CD/CBD/EtOH
	15% solid content	20% solid content	15% solid content
Recovery dose (μg)	2435.00 ± 187.79	2795.14	1646.80 ± 53.22
Recovery fraction (%)	89.00 ± 0.42	81.71	94.70 ± 1.25
Emitted dose (μg)	2232.45 ± 217.94	2633.35	1454.00 ± 31.05
Emitted fraction (%)	91.55 ± 1.87	94.21	88.45 ± 4.78
FPD (μg)	1502.70 ± 123.35	1892.11	935.30 ± 151.76
FPF (%)	61.70 ± 0.28	67.69	57.00 ± 11.09
Deep delivered CBD/capsule (μg)	125.23 ± 10.282	157.68	77.94 ± 12.65
MMAD (μm)	3.83 ± 0.06	3.62	4.01 ± 0.55
GSD (-)	2.02 ± 0.01	2.03	1.98 ± 0.10

CBD – Cannabidiol; HP-β-CD – Hydroxypropyl-beta-cyclodextrin; EtOH – Ethanol; FPD – Fine particle dose; FPF – Fine particle fraction; MMAD – Median mass aerodynamic diameter; GDS – Geometric standard deviation. Values are expressed as mean ± SD. n = 2 (except in powder of 20% HP-β-CD/CBD, which was one sample)



**Figure 5- Aerodynamic particle size profile of CBD of the three spray dried powders.**

## 5 Discussion of results

An initial experiment was carried out with the primary aim of characterizing the particles generated from a straightforward formulation comprising hydroxypropyl-beta-cyclodextrin (HP- $\beta$ -CD) and cannabidiol (CBD) (See section 5.1). To delve deeper in this investigation, the study expanded to assess the influence of two key variables: a modification in composition involving the addition ethanol, and a variation in quantity through an increase in the solid content of the spray dried solution.

Subsequently, to comprehensively assess particle deposition in the pulmonary tract, an *in vitro* analysis of the aerodynamic particle size distribution was conducted (See section 5.2). This analysis encompassed the examination of the three distinct types of produced powders, with the aim of establishing correlations between particle properties and their respective pulmonary deposition profiles.

### 5.1 Formulation impact on powder's properties

The characteristics of an inhalable spray dried powder containing CBD have never been defined previously. Understanding these properties is vital for optimizing formulations with higher pulmonary deposition and enhanced aerosolization. A basic formulation was conducted utilizing HP- $\beta$ -CD and CBD to uncover the fundamental characteristics of the powder. To overcome the challenge of CBD's limited water solubility and increase its solution concentration, an alternate formulation employing ethanol was devised.

The selection of excipients was informed by prior research. HP- $\beta$ -CD was chosen for inclusion in the dry powder inhaler (DPI) formulations due to their well-documented effectiveness in mitigating the low water solubility of drugs through the formation of cyclodextrin-drug complexes (47). Furthermore, HP- $\beta$ -CD has demonstrated the ability to enhance drug molecule permeability and bioavailability (48). HP- $\beta$ -CD has exhibited safety *in vitro*, specifically in human airway epithelial Calu-3 and A549 cells. Consequently, its incorporation in the formulation of DPI for inhalation is deemed safe from a safety perspective (49).

The additional excipient, ethanol, has been studied in prior studies for its role in spray dried solutions. It has been shown to contribute to the formation of powder particles with a wrinkled morphology, rendering them well-suited for pulmonary administration. This specific

particle structure is advantageous for efficient delivery to the lungs, aligning with the objective of achieving optimal pulmonary administration in our formulation (50). In terms of safety, this compound has demonstrated pulmonary tolerance at concentrations of up to 10% (51).

### **5.1.1 Spray dried particle's properties**

The solutions were prepared (See Section 3.2). Subsequently, atomization was employed, following the specified spray drying parameters. The resulting powders were then subjected to several tests to analyze their properties. As presented in Table 3, the process yields across all formulations were notably high, ranging from 55 to 69%. Yield loss was due to powder adhesion to the cyclone and drying chamber walls. This highlights that the resulting powder exhibited moderate stickiness, reaffirming the appropriateness of the spray drying method for the efficient production of this specific product. It's important to note that the reported value of 55% for the formulation with 15% solid content HP- $\beta$ -CD/CBD considers an operational issue during the process where a portion of the solution was inadvertently wasted. If we accurately account for the two correct vials, the process yield should be revised to 61%.

Regarding particle size, all formulations exhibited sizes within the range of 1 to 5  $\mu\text{m}$ , suggesting their capability to reach the deep pulmonary tract effectively. The results also indicate that solutions with higher solid content might yield larger particles, implying a potential direct correlation between solid content and particle size. Based on the lowest span value obtained, it is plausible to conclude that the HP- $\beta$ -CD/CBD solution with a solid content of 20% exhibits lower polydispersity in comparison to the other formulations.

The determined water content values ranged from 3.52% to 5.22%, with the highest moisture content observed in the spray dried solution incorporating ethanol. It's evident that higher moisture levels can impact the flowability of the powder by promoting the formation of liquid bridges. Conversely, excessively low water content could indicate an unstable product due to hygroscopicity. Thus, identifying an optimal water content value remains an ongoing endeavor, striking a balance between adequate flow properties and product stability. (52).

Regarding the measurements of tapped and bulk density, an initial and singular assay was conducted on a sample of 15% HP- $\beta$ -CD/CBD. As anticipated, the results revealed that the powder exhibited poor flowability, with a Hausner ratio of 1.57. This is attributed to the

microsize of the particles, resulting in limited air space between them and increased cohesive forces (53). Due to the significant quantity of powder required to conduct this analysis and the consistency in expected poor flowability across formulations, further assays were deemed unnecessary for the remaining samples.

Ethanol quantification was not conducted in the current study; however, it is acknowledged that for subsequent investigations, quantifying ethanol in the powders produced from stock solutions containing ethanol is essential. It is imperative to ensure that the ethanol content in these powders adheres to the recommended limit of below 0.5%, as specified in the ICH Q3C (R6) guideline (54).

### **5.1.2 Morphology of spray dried particles**

In terms of particle morphology, Table 4 illustrates the key aspects analyzed in samples from each formulation using scanning electron microscopy (SEM). Across all formulations, atomized particles exhibited distinctive dimples, indicating a wrinkled surface. Such wrinkled morphology is commonly observed in spray dried particles designed for lung administration and has been associated with improvement of aerosolization (55). This observation was further substantiated by complementary topographic photographs presented in Figure 4.

Interestingly, an inverse correlation was observed between the number and depth of dimples. Formulations with lower solid content displayed a higher number of pronounced particle dimples. Additionally, ethanol was found to induce more pronounced dimpling in the particles, although these variations were not statistically significant. It is evident from the comparable deflation and correlation ratios observed in particles produced with and without ethanol, that this substance did not markedly alter the particle morphology.

Notably, the most deflated yet corrugated particles were observed in formulations produced with higher solid content feeding solutions, indicating. This suggests that the quantity of solid content may indeed alter the particle's morphology, emphasizing the importance of solid content in shaping particle characteristics (56,57).

To assess the potential impact of the spray drying process on CBD degradation, samples were extracted from the solution post-complexation to quantify the CBD content. Similarly, to determine the CBD concentration in the resulting powders, samples were extracted post-atomization and subjected to the same quantification method.

### **5.1.3 CBD quantification in spray dried powder**

Contrary to initial expectations, ethanol was found to adversely affect the solubility of CBD in the solution compared to a medium using only water, as outlined in Table 5. It suggests that a possible competition between ethanol and CBD can occur, indicating that the formation of EtOH-(HP- $\beta$ -CD) was more prevalent than the formation of supramolecular complexes composed of CBD-EtOH-(HP- $\beta$ -CD) (58). Moreover, higher solid content in the feeding solution yielded increased CBD concentration up to 2.02 g/L. This can be attributed to the higher quantity of solubilized HP- $\beta$ -CD, leading to enhanced complexation of CBD (59).

As anticipated, an increase in the quantity of CBD in the solutions correlated with a higher quantity of CBD in the resulting spray dried powders. The highest concentration, reaching 9.5 mg/g, was observed in powders without ethanol and with a 20% solid content. This outcome opens avenues for further investigation into whether higher solid content continues to yield higher concentrations of CBD.

Due to the drug recovery value of 118.36% observed in the 15% HP- $\beta$ -CD/CBD samples, an additional 50 mL solution was independently produced and subjected to atomization to ascertain its recovery value. The subsequent result of 98.65% was within an acceptable range and helped explain that the small sample quantities used initially may have contributed to minor errors in the recovery calculation.

Overall, the recovery rates for CBD were nearly 100%, indicating that the quantity of CBD in the stock solution closely aligns with the quantity in the powder. This suggests that the spray drying process is safe in terms of CBD degradation.

## **5.2 Powder's pulmonary deposition behavior**

The aerosolization performance was assessed using a cascade impactor, due to its unique features. This equipment separates samples based on particle inertia, a parameter determined by velocity and aerodynamic particle size, without the need to ascertain particle density or shape. This approach allows for a comprehensive understanding of particle behavior in a moving air stream through aerodynamic particle size distribution (APSD).

In this analysis, a Next Generation Impactor (NGI) was employed, where samples of powders from each formulation were subjected to the procedure detailed earlier (see Section 3.10). Aerosolization efficiency by different dimensions obtain from analysis of NGI data that are summarized in Table 6.

Recovery aspects of the assay were calculated based on the ratio of the quantity of powder initially placed inside the capsules to the total powder detected across all parts of the equipment. These results indicate that operational losses were not significant, with values ranging from 81.71 to 94.66%.

The emitted fraction is determined as the disparity between the recovery fraction and the remaining powder in the capsules, device, and mouthpiece. In essence, it quantifies the powder that successfully exited the device, reaching the throat and subsequent stages of the equipment. The obtained values were remarkably close, indicating comparable capabilities in reaching the pulmonary airways. The minimum observed emitted fraction was 88.43%, while the maximum recorded was 94.21%.

The highest emitted fraction was observed in the 20% HP- $\beta$ -CD/CBD powder, implying a potential correlation that increasing solid content leads to a higher emitted fraction. Given that this powder exhibited a higher deflation ratio, this morphology could positively influence the powder's performance in exiting the inhaler.

The fine particle fraction (FPF) represents the proportion of powder that successfully reaches and deposits in stages 2 to 8. In simpler terms, it signifies the quantity of powder with particles smaller than 5  $\mu\text{m}$ , allowing them to reach the deep pulmonary tract.

The fine particle fraction results ranged from 57.0% to 67.7%, which are considered high compared to other findings (60). Consistent with the emitted fraction, the powder produced with a feedstock of 20% HP- $\beta$ -CD/CBD exhibited the highest value of 67.69%, which converted to CBD quantity that reaches the deep lung zone is 157.68  $\mu\text{g}$  per capsule. This indicates that it possesses the most favorable properties for deposition in the deep parts of the lungs. Furthermore, ethanol appears to have a detrimental effect on powder aerosolization properties, while higher solid content seems to enhance it.

The MMAD values ranged from 3.62 to 4.01  $\mu\text{m}$ , falling within the acceptable range for respirable particles (1–5  $\mu\text{m}$ ). Furthermore, the GSD values for all spray dried powders were consistently below 2.03, indicating a narrow size distribution predominantly centered

around a fine particle size. This observation suggests an efficient pulmonary delivery, as a tight size distribution enhances aerosol penetration into the respiratory tract (61).

Using the gathered data on the quantity of CBD detected in each part of the impactor, a graph in Figure 5 was generated to illustrate the aerodynamic profile of the particles for each spray dried powder.

## 6 Conclusion

In this study, we embarked on the development of an inhalable powder aimed at achieving deep lung deposition. To better comprehend the effects of various formulation modifications, a series of tests were conducted. Our findings underscore the crucial role of hydroxypropyl- $\beta$ -cyclodextrin (HP- $\beta$ -CD) in enhancing the solubility of cannabidiol (CBD), thus enabling the production of powders with higher concentrations of the drug. Notably, the addition of ethanol did not yield a significant improvement in CBD solubilization.

Furthermore, the spray dried particles exhibited favorable aerosolization properties, indicating their suitability for effective lung delivery. This, in turn, enhances the therapeutic efficacy of the inhalable powder. Additionally, the process of spray drying proved to be well-suited for the production of this powder, ensuring consistent and reproducible characteristics of the powder while causing minimal degradation to the final product.

To enhance the confidence and reliability of our findings, it is advisable to expand the number of samples in future experiments. This can provide a more comprehensive understanding of the performance and attributes of the inhalable powder.

Finally, while promising results have been achieved, it is essential to conduct further investigations to determine the solid content level at which particle properties remain optimal and suitable for lung administration. This will help to define the boundaries of feasibility and efficacy in this promising area of research.

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