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**INHALABLE CANNABIDIOL**  
**FORMULATION CONSIDERATIONS FOR EXTREMELY**  
**LIPHILIC, UV-LABILE SUBSTANCES**

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**DISSERTATION**

**IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF**  
**DOCTOR OF NATURAL SCIENCES**  
**OF THE FACULTY OF MATHEMATICS AND NATURAL SCIENCES**  
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*“Share your knowledge.*

*It is a way to achieve immortality.”*

-Dalai Lama XIV

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**Für meine Familie:**

**Ulli**

**Dieter**

**Philipp**

**Rieke**

**& Teddy**

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# ***1 INTRODUCTION AND OBJECTIVES***

## ***1.1 INTRODUCTION***

Cannabis and its phytochemicals have been used for medicinal purposes for more than 5000 years. However, cannabis has been shown to create various health problems. This and the increase of recreationally used, or rather abused, cannabis resulted in the prohibition of the drug [1].

Cannabis is the most commonly used illegal drug in Germany. In 2015, 16.3 % of young adults have consumed cannabis at least once during the preceding 12 months. It is estimated that about 0.5 % of the German population aged between 18 and 64 years fulfil the criteria of cannabis addiction [2].

Today, more and more states are starting to liberalise cannabis or at least allow the prescription of cannabinoid-based medicines. For example, Canada legalised cannabis for medicinal use in July 2001 (Marihuana Medical Access Regulations) and even for recreational use (for adults) in October 2018 (federal Cannabis Act). According to the National Organisation for the Reform of Marijuana Laws (NORML), eleven of the United States of America have completely legalised cannabis and only three states (Idaho, Nebraska & South Dakota) do not allow the medical use of cannabis or cannabidiol. Germany has legalised cannabis for certain medical contexts in March 2017 (Law "*Cannabis als Medizin*"). Cannabis (marijuana, plants & plant parts) and dronabinol (synthetic  $\Delta^9$ -Tetrahydrocannabinol; THC) are also included in annex III of the *Betäubungsmittelgesetz* [3] (narcotics law) whereas cannabidiol (CBD) is completely legal and marketed in food supplements (e.g. Nordic Öl). However, several articles, which were published in the second half of July 2020, reported that the European commission delayed 50 CBD-related novel food applications through September to consider the question of whether CBD extracted from the plant should be considered a narcotic.

Research on isolated cannabinoids did not start before the late 1930s and the pharmacological studies for the main compounds were first published in the 1960s [4] (THC) and 1970s [5] (CBD). It is unknown if the pharmacological effects of isolated cannabinoids are comparable to plant extracts with high contents of said phytochemicals.

Cannabinoids have been associated with a multitude of effects (e.g. anxiolytic, anti-inflammatory (see 3.1.1)) [1], and administration via several routes has been investigated: pulmonary (smoking, vaping), oral, oromucosal, rectal, transcutaneous and intravenous [6], with smoking, of course, being the principal route of cannabis administration. This route is rapid and efficient in delivering the drug to the central nervous system (CNS), which in turn contributes

to the abuse potential. However, smoking is one of the main reasons for health issues associated with cannabis [7].

The high number of effects associated with cannabinoids show their tremendous pharmaceutical potential. However, the obvious connection between cannabis and the inhalative route has not yet yielded any internationally marketed medical products. However, local markets (e.g. Israel) have some products (e.g. Syqe<sup>®</sup> inhaler). This might be due to different jurisdiction or formulation difficulties associated with cannabinoids, which can be of pharmacological or physicochemical origin.

Consequently, this thesis presents a possible approach in the development of a cannabinoid-containing formulation for pulmonary application. This formulation type combines the advantages of the pulmonary application route over the oral route while minimising the drawbacks of smoking in general.

## *1.2 OBJECTIVES*

The lack of inhalable formulations containing an isolated cannabinoid shows the need for studies in this area. CBD was chosen as a model substance for this work as, unlike THC, it could be used without restrictions during this project.

Recreational pressurised metered dose inhalers (pMDIs; e.g. Mystabis™, Quest Aero™ inhaler™), which contain cannabis extracts, are the only marketed products that have a typical medical inhalation device. Based on these products pMDIs, which contain the model drug, were produced and characterised.

Several publications state that some cannabinoids (e.g. THC & CBD) are thermo- and/or photolabile [1,8], it was deemed necessary to further investigate the stability of the model drug and to possibly find a suitable protecting agent.

The development of a suitable inhalable formulation needs to meet certain requirements. These are relatively high doses (see chapter 3.1.1), an aerodynamic particle size of 0.5 – 5 µm as well as a sufficient stability of the model drug. Due to the high required doses, the formulation of an interactive blend appears to be unfeasible. Whereas the physicochemical properties of the API (active pharmaceutical ingredient) could impede the generation of inhalable CBD particles. Hypothetically, this means that the formulation of engineered particles is the most auspicious.

Due to the physicochemical properties of the model drug (see chapter 3.1.1), spray-drying was chosen to prepare the formulations, which were subsequently characterised for several properties (e.g. particle size, hygroscopicity). Due to the stability issues of the model drug, the formulations were subjected to a twelve-week stability study at three different conditions and in different packaging. After the stability study, the most important properties (e.g. API content, aerodynamic performance) of the formulations were re-characterised. This enabled the determination of the optimal formulation while also suggesting optimal packaging.

## 2 THEORETICAL BACKGROUND

### 2.1 PRINCIPLES OF PULMONARY APPLICATION

#### 2.1.1 ANATOMY AND PHYSIOLOGY OF THE HUMAN RESPIRATORY TRACT

The physiological purpose of the lung is the uptake of oxygen while simultaneously emitting carbon dioxide, the final product of the oxidative metabolism [9].

The main entrance of the respiratory tract is the nose. Here the inhaled air is warmed, humidified and cleaned [9]. The airways continue through the oropharynx and trachea before arborising numerous times (see Figure 2–1). Therefore, the entirety of bronchial system is often referred to as the bronchial tree [9].

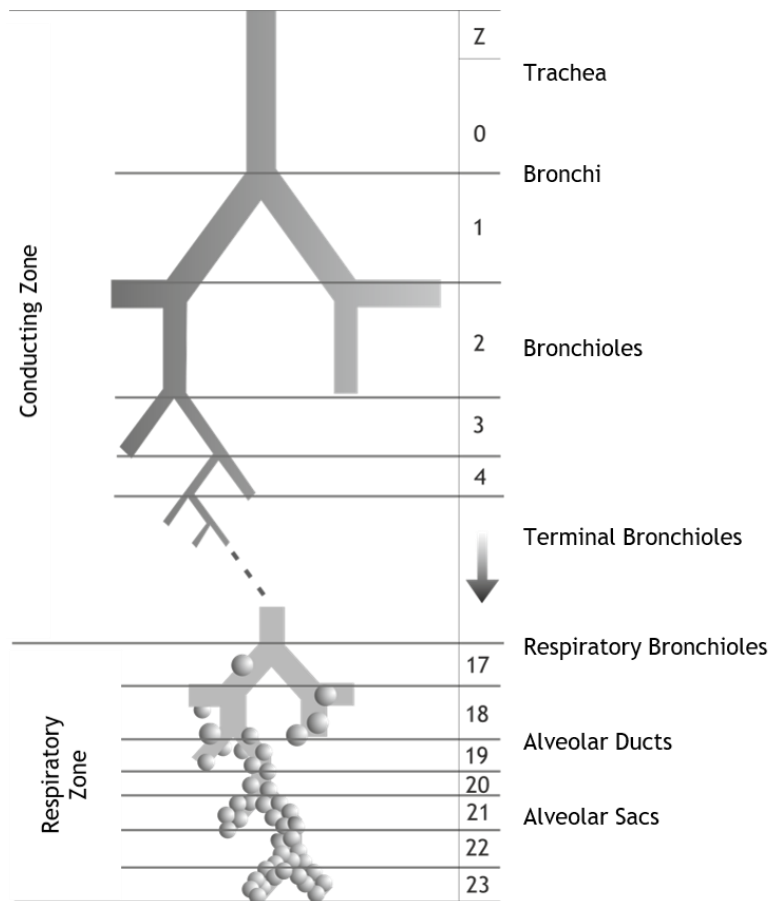


Figure 2–1: Weibel "A" model (z = number of arborisations), adapted from [10]

The diameter of the airways shrinks after every arborisation, this results in a diameter of about 200 – 300  $\mu\text{m}$  for the alveoli [9–11] (or alveolar sacs [11]). It is estimated that the human lungs contain circa 300 million alveoli, which have an overall surface of 80 – 90  $\text{m}^2$  [9,11].

Despite its name, the respiratory epithelium is not responsible for the gas exchange but the cleaning of the lungs [11]. It is characterised by cilia which transport mucus and inhaled particles [9,11] out of the lungs and is therefore exerting a protective task. Towards the terminal end of the bronchial tree the epithelium becomes flatter and loses the cilia before transitioning into the alveolar epithelium [9] in the alveoli, where the gas exchange takes place. The gas exchange is driven by a difference in partial pressure of the respective gas and the oxygen binding capacity of haemoglobin [9] and is conducted in the capillary membranes of the alveoli.

### *2.1.2 THERAPEUTIC GOALS OF PHARMACEUTICAL AEROSOLS*

The lung has numerous properties, which makes it an intriguing target for both local and systemic drug delivery. The large surface area and the very thin alveolar epithelium, which is very well supplied with blood, enable rapid drug uptake [10,12–14], with the uptake velocity being mainly dependent on the molecules' lipophilicity and its diffusivity (linked to molecular size) [13]. The low number of metabolic enzymes make the lung an intriguing option for application of peptides and proteins [10,12,14]. Additionally, the enterohepatic circulation and the first pass effect can be circumvented, as the lung is not connected to the portal vein.

Drugs administered for local treatment are mostly used in diseases like asthma or COPD (e.g. anticholinergics, corticosteroids, sympathomimetics [9]), or local infections (e.g. antibiotics in the treatment of pneumonia or tuberculosis) of the lung, which could be linked to other diseases like cystic fibrosis [15]. An advantage of locally treated pulmonary diseases is that the overall dose can be reduced [16], this leads to a lower systemic exposure and therefore less toxicity [15].

The focus of recent studies on pulmonary application has shifted to the systemic drug delivery [16], especially of peptides and proteins. Proteins which have been evaluated for their systemic application via the lung include insulin, calcitonin, thyroid-stimulating hormone, follicle-stimulating hormone and different growth hormones [12].

Peptides and proteins are normally administered in chronic treatments and via an injection, which often results in poor compliance [12]. This makes the pulmonary delivery, as a non-invasive alternative, especially interesting [12]. Insulin was the first inhalable protein to be marketed when it was approved in 2006 (Exubera®). However, Exubera® was discontinued in 2007 for economic reasons [17].

Small molecules which are evaluated to be administered pulmonary for systemic effects include fentanyl or other opioids for (cancer) pain [18] and phosphodiesterase type 5 inhibitors for pulmonary hypertension and sexual dysfunction [19].

## Principles of Pulmonary Application

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Newer research addresses the administration of vaccines to the respiratory tract [20]. This is especially interesting for all pathogens that enter the body via this route [21]. Nasal and pulmonary administration of vaccines are much more effective in generating a mucosal (local) immune response, compared to intramuscular injection, while also generating a systemic immune reaction. This means that both IgA and IgG antibodies result from this type of vaccination.

The significant implementation in today's health system and the intriguing potential for future uses explains the rising significance of pharmaceutical aerosols.



## 2.2 FUNDAMENTALS OF PULMONARY INHALATION

An aerosol, a gas-borne suspension of liquid or solid particles [11], is needed to deliver drugs to the lung. The ability to reach certain depths of the lung is mainly dependent on the aerodynamic diameter.

$$d_{aer} = d_g \cdot \sqrt{\frac{\rho}{\rho_0 \cdot \chi}} \quad (2-1)$$

$d_{aer}$	Aerodynamic diameter	$[\mu m]$
$d_g$	Geometric diameter	$[\mu m]$
$\rho$	Particle density	$[g/cm^3]$
$\rho_0$	Sphere with a density of $1 g/cm^3$ and a volume equivalent to the particle	
$\chi$	Dynamic shape factor	

Equation (2–1) reveals that the aerodynamic diameter can be decreased by reducing the particle size (geometric diameter) or density (e.g. higher porosity) or by using a particle collective with a higher dynamic shape factor [22].

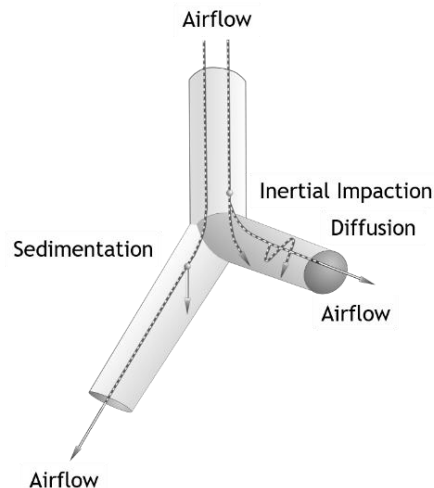


Figure 2–2: Main deposition mechanisms, adapted from [10]

To reach the desired parts of the lung an aerodynamic diameter of  $0.5 - 5 \mu m$  is needed [14]. The aerosolised particles will deposit in the lungs by impaction, sedimentation or diffusion. Impaction is the inertial deposition of a particle onto a surface [14,23] and is the typical way of deposition for particles  $> 5 \mu m$ . These will normally deposit in the larger airways, where high inertial forces are created by the higher air flow velocity and the bifurcations of the bronchial tree [10,14]. Sedimentation is linked to the gravitation and therefore increased by

higher particle mass and longer residence but decreased by a high air exchange (faster breathing [10]). It is the main deposition mode of particles in the desired size range (0.5 – 5  $\mu\text{m}$ ) and will normally occur in the smaller conducting airways due to decreasing air velocities [14]. Diffusion, or Brownian motion, is the acquisition of random motion caused by the impact of air molecules [11,14]. It is a time-dependent process and increased with decreasing particle sizes, meaning particles  $< 0.5 \mu\text{m}$  will mainly deposit through diffusion [10,14]. However, due to the long dwell time in abeyance these particles rarely deposit in the respiratory zone and are often exhaled [22]. The deposition mechanisms are shown in Figure 2–2.

Four types of inhalation devices are commonly used nowadays [24]:

1. Nebulisers
2. Pressurised metered-dose inhalers (pMDIs)
3. Non-pressurised metered-dose inhalers (i.e. soft mist inhalers (SMIs))
4. Dry powder inhalers (DPIs)

The following subchapters will outline the differences between the four types of inhalation devices.

### *2.2.1 NEBULISERS*

In nebulisers one of three different principles to achieve aerosolisation of liquid preparations is commonly utilised. Two of which use piezo crystals (ultra-sonic and (vibrating) mesh nebulisers), while the third one utilises pressurised air (jet nebuliser). Only liquid formulations (dispersion (marketed products), emulsion, solution) can be dispersed with nebulisers. After generation, the aerosol is inhaled via a mouthpiece or a facemask [25].

Ultra-sonic nebulisers transfer the vibration, which is generated by the piezo crystal, directly to the formulation, this causes the formation of aerosol droplets on the surface of the liquid. Membrane nebulisers, in contrast, use their piezo crystal to oscillate a perforated membrane, which then separates the preparation into inhalable droplets. In jet nebulisers the formulation is passed through a nozzle and subsequently torn apart by compressed air [25].

As the production of an inhalable aerosol is completely independent from the patient, nebulisers are especially suitable in the therapy of those who are not able to use a specific inhalation technique (e.g. infants, patients suffering from dementia, ventilated or unconscious patients) or patients who are unable to generate a sufficient volumetric air flow (e.g. children, elderly, asthma/COPD patients) to disperse a formulation (needed in DPIs, see 2.2.3).

Although nebulisers can deliver large doses of API, they also exhibit some disadvantages. A large amount of residual can often be found in the device. As inhalation with most nebulisers

is a prolonged procedure with a large, power dependent device, some of the active will be exhaled, meaning that only a fraction of the formulation reaches lungs [26]. This results in a low efficiency [27].

The main drawbacks of liquid formulations are the susceptibility to chemical degradation [28] and the possibility of extracting potentially harmful compounds from the primary packaging. Additionally, water-containing formulations are also more susceptible to microbiological degradation.

As a result, nebulisers only have a secondary role in today's inhalation therapy.

## *2.2.2 METERED DOSE INHALERS*

### *2.2.2.1 Pressurised Metered Dose Inhalers*

PMDIs, like Nebulisers, are devices which provide aerosolisation of liquid API formulations, normally dispersions or solutions. The peculiarity of pMDIs is that the formulation contains a propellant. Until the late 1980s chlorofluorocarbons (CFCs) were the propellants of choice but as they are a significant threat to the environment, the Montreal Protocol (signed in 1987) called for their elimination since 1996. Hydrofluoroalkanes (HFAs) subsequently superseded CFCs as the main propellants [29]. However, newer developments show that HFAs also pose a significant threat to the climate and were retrospectively added to the Montreal Protocol via the Kigali Amendment on 15 October 2016. Article 2J of the Kigali Amendment came into effect on January 1<sup>st</sup>, 2019, which means that the use of HFAs must decrease in the coming years [30]. Isobutane and HFA 152a are investigated as a potential alternatives to the traditional HFAs in inhalers [31,32].

PMDIs are the oldest of the modern portable inhalation devices, as the first product reached the market in 1956 (Medihaler<sup>®</sup>) [29]. The formulation is kept in a closed container with a metering valve, which volumetrically ensures the emission of uniform doses. After leaving the can, the formulation is led through a spray orifice, which leads to the generation of an aerosol [33]. Due to the rapid evaporation of the propellant, solid particles in the inhalable range (0.5 – 5 µm) are formed and then inhaled by the patient.

Advantages of pMDIs include the portability of the device, a large amount of doses, a short inhalation procedure, which makes it especially suitable for emergency medications (e.g. exacerbation of asthma), and relatively low costs [25,27,34].

Disadvantages include the mandatory coordination of inhalation and actuation, which can be problematic for certain patients (compare with 2.2.1) and the inability to deliver high doses (rarely > 1 mg) [34]. The general disadvantages of liquid formulations apply to pMDIs as well.

If the inhalation manoeuvre is not performed with the adequate technique, a large amount of the formulation will deposit in the oropharynx, the primary reason being the high exit velocity of the aerosol from the device [33]. This can lead to adverse effects (e.g. oral candidiasis with glucocorticoids [35]) or unpleasant sensations like the Cold Freon® effect (evaporation chill of remaining propellant on the tissue of the oropharynx; incidence lower with HFA pMDIs) [27,34].

The problems resulting from the high exit velocity can be reduced with the use of a spacer [25,34]. A spacer is an auxiliary, which is fitted to the mouthpiece of the pMDI. The aerosol is slowed in the cavity of the spacer [35] and can subsequently be inhaled (in multiple breathes if the spacer contains an appropriate valve) without impaction in the oropharynx. Due to the additional cost, however, spacers are normally only provided for infants or elderly people who have problems with the correct inhalation technique.

Newer pMDI devices include the Autohaler® which is breath-actuated and releases the aerosol as soon as the patient reaches an inspiratory flow of 30 L/min [25].

Nonetheless, pMDIs are very important in today's inhalation therapy. This is also evidenced by their market share in several countries (Figure 2–3).

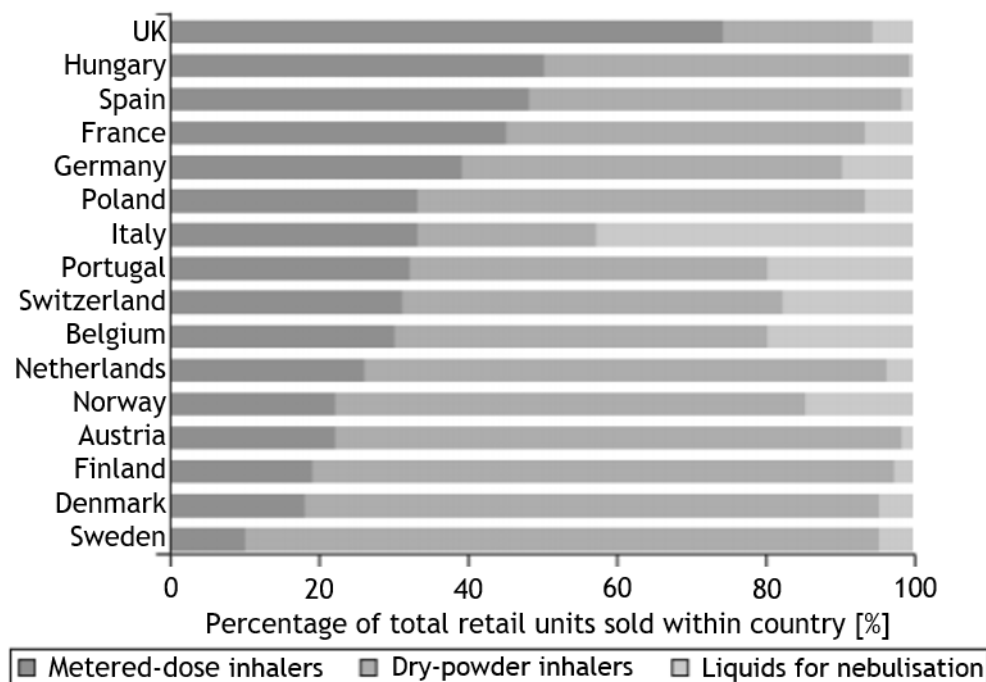


Figure 2–3: Market shares of inhalers in European markets (2011) [36]

As this type of inhaler is important in the recreational use of cannabis, it was also investigated over the course of this project.

### 2.2.2.2 *Soft Mist Inhalers*

SIMs are a special kind of metered dose inhalers as they do not rely on a propellant or electrical energy (like nebulisers) but mechanical energy, provided by a spring, to generate the aerosol [37]. Boehringer Ingelheim first established the technology with the approval of the RespiMat® in 2004. Up to date SIMs are only able to aerosolise solutions.

The RespiMat® forces the formulation through a system made up by filter channels and two nozzles [37]. The two emerging liquid jets impact in a defined angle resulting in the dispersion of the formulation. Due to the setup of the dispersion system, the aerosol exits the device at a considerably slower velocity than in pMDIs while the cloud also persists longer (about 1.5 s) [37].

As SIMs, like pMDIs, require a coordination of actuation and inhalation, a source for potential errors is inherited from their technological predecessors. Due to the lower velocity and longer duration, however, this coordination could be easier [38].

Advantages of SIMs are the reduced deposition of API in the oropharynx, due to the low exit velocity, and a small size, which makes them suitable for emergency medications (e.g. Berodual® RespiMat®). The device itself, however, is relatively expensive [25].

The RespiMat®, which was originally designed as a non-reusable device, has since been made reusable (April 2019; up to six cartridges). Other companies (e.g. Merx, MRX004) have also started to market generic SIMs.

### 2.2.3 *DRY POWDER INHALERS*

DPIs initially reached the market with the approval of the capsule based Spinhaler® in the UK in 1967 [29]. Capsule based devices are considered as single dose DPIs. In multi-dose DPIs the formulation is stored in blisters (stripes (e.g. Diskus®) or disks (e.g. Diskhaler®)) or in a reservoir. The first approved multi dose DPI was the Turbohaler®, a reservoir-based inhaler introduced by AstraZeneca in 1988 [29]. Newer developments in DPI research even include single use inhalers like the Twincer® device.

As the name states, DPIs are used to aerosolise solid, powdery formulations. In contrast to nebulisers, pMDIs and SIMs, DPIs rely on the inhalative airflow to disperse the formulation [29]. This eliminates the need to coordinate inhalation and actuation [25] but introduces the disadvantage of the inhalable fraction being directly dependent on the achieved airflow [39]. This means that DPIs are unsuited for patients whose inspiratory flow is impaired, which is often the case in severe Asthma or COPD [40]. The amount of drug which ultimately reaches the lungs depends on the formulation's properties, the device and the achieved inspiratory flow [27,41]. In dry powder inhalation the aerosolisation is usually passive as active devices

like the Spiromax<sup>®</sup> were not able to assert themselves due to being complex and therefore expensive and vulnerable to failure [42].

Like SMIs and pMDIs, DPIs are small devices, however, they have the advantage of being suitable for the inhalation of higher doses [25] which is especially interesting for antibiotics [41] and for example realised with the TOBI<sup>®</sup> Podhaler<sup>®</sup>. Here each capsule contains 28 mg of tobramycin (ca. 50 mg of total formulation) which is significantly more than the normal upper limit of pMDIs (see 2.2.2.1) [43].

Substances normally exhibit a higher stability in a solid form and the possibility of extracting compounds from the inhaler is also negligible [44].

In contrast, however, DPI formulations are much more vulnerable to exogenous factors (e.g. humidity and rapid, large temperature changes) [25,27,41] than pMDIs. These exogenous factors are known to affect the disaggregation of the formulation, which in turn results changes of the efficiency of the pulmonary drug delivery. This is the main reason for DPIs being generally more susceptible to issues with the dose uniformity [41].

Three different types of formulations are generally used in DPIs: Interactive blends, soft pellets and engineered particles (Figure 2–4). Interactive blends are the most common type of DPI formulation.

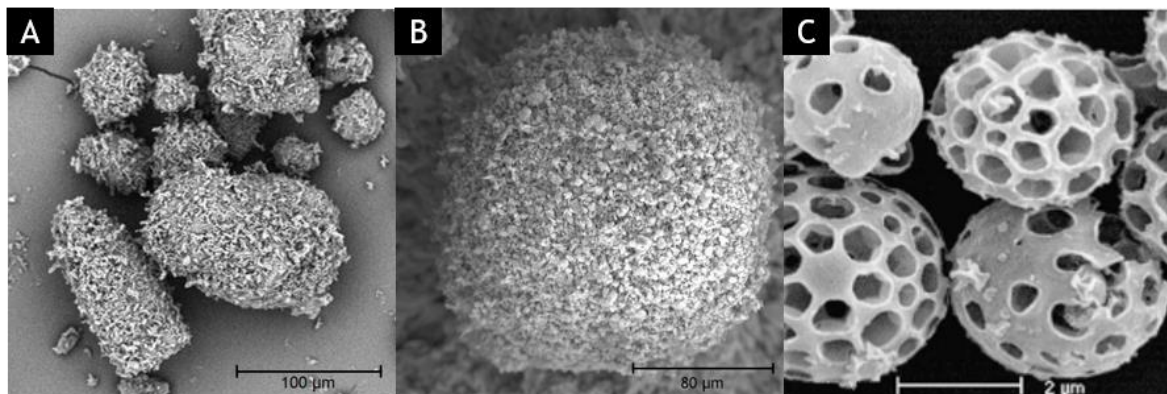


Figure 2–4: SEM images of an interactive blend (A), a soft pellet (B) and Pulmospheres™ [45,45] (C)

An interactive blend (Figure 2–4A) is characterised as a mixture of larger carrier particles (50 – 200 μm, often Lactose [25,27,42]) and the API (binary blend), which is micronised due to the size requirements (0.5 – 5 μm, see chapter 2.2), and possibly further excipients (ternary blend) like magnesium stearate or so-called fines, which can be a micronised quality of the carrier material. Magnesium stearate can, for example, increase the overall flowability of the formulation while also reducing the susceptibility to humidity [46,47]. The addition of fines

can enhance the performance of a DPI formulation by increasing the fine particle fraction (FPF) [48].

After mixing in an appropriate blender, the API will adhere to the surface of the carrier [42] (ternary blends are normally prepared by multiple mixing steps). The mixing process will break up API agglomerates into primary particles. These primary particles will primarily adhere to the carrier surfaces, as the probability to collide with the carrier surface is much higher than a collision with other drug particles. As the adhesion forces between carrier and API are lower than the cohesion forces of the API, a larger amount of the primary drug particles will be dispersed from the formulation and thus reach deeper compartments of the lung.

Interactive blends normally contain between 0.1 – 4 % of API [49]. This also means that the dosing of the formulation is mainly dependent on the flowability of the carrier [27,50], which is generally very good due to the large size of the carriers. The low amount of drug, however, also means that interactive blends are unsuited for high-dose inhalation [51].

Controlled agglomerates of pure (micronised) API or a combination with equally sized excipients are called soft pellets (Figure 2–4B) [51,52]. During production, soft pellets will be rounded off which, in combination with their relatively large size, ensures a good flowability. The mechanical instability ensures a good dispersibility [51]. As these formulations can be made up of pure drug, soft pellets are suited for high-dose inhalation [52]. The Turbohaler® was specifically developed for the administration of soft pellets.

Engineered particles are specially tailored or modified. One formulation approach is represented by the PulmoSphere™ technology [52] (Figure 2–4C) which is used in the aforementioned TOBI® Podhaler®. PulmoSpheres™ are compound particles which are characterised by their very porous shape [53]. They are produced by a sophisticated spray-drying procedure from a hydrophilic emulsion [52,53]. A distinct advantage of this technology is the reduced dependency on the inspiratory flow rate needed to disperse these porous particles [53].

Another example of engineered particles was introduced with the Technosphere® technology, which was used in an insulin formulation (Afrezza®) [42]. This technology utilises fumaryl diketopiperazine particles generated through acid induced crystallisation [17]. These crystals can adsorb insulin during the precipitation [17], making it pulmonary available [42]. However, Afrezza® has since been withdrawn from the market in 2016 [54].

Depending on the type of engineered particle, it is possible to generate a formulation which has an API content of more than 90 wt% making these formulations suitable for high-dose inhalation [53].

Due to the importance for this work, strategies for substances with formulation difficulties, which often result in the production of engineered powders, are more closely described in chapter 2.4.



## 2.3 CANNABINOIDS

Cannabinoids are substances which interact with the receptors of the so called endocannabinoid system and can be divided in two groups: Endogenous and exogenous cannabinoids [55].

The endocannabinoid system was discovered in the early 1990s when the psychotropic effects of THC were investigated [55,56]. As THC is a substance from cannabis plants, this complex signalling system was denoted as the endocannabinoid system [56]. Originally, two G protein-coupled receptors were discovered (CB<sub>1</sub>R and CB<sub>2</sub>R). The CB<sub>1</sub>R is predominantly localised in the CNS and can for example be found in the basal ganglia, the hippocampus, the cerebellum and the cerebral cortex, whereas the CB<sub>2</sub>R is predominantly found outside of the CNS, for example in the spleen, and can be up-regulated during injury and inflammation in sensory neurons or the spinal cord [57]. Their most studied endogenous ligands (anandamide (Figure 2–5) and 2-arachidonoyl-sn-glycerol (2-AG)) were subsequently labelled as endogenous cannabinoids [55,56]. Both are lipid-based retrograde neurotransmitters and have the ability to modify the sensitisation of nociceptive stimuli [58].

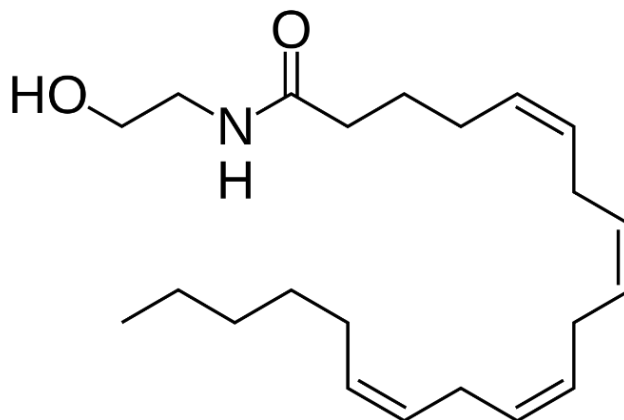


Figure 2–5: Structural formula of anandamide, adapted from [59]

Cannabis plants contain more than 80 different phytochemicals, which are the exogenous cannabinoids. However, not all of these interact with the receptors of the endocannabinoid system. In fact, only THC and  $\Delta^9$ -tetrahydrocannabivarin (THCV) exhibit a high affinity to both receptors [56]. Several phytocannabinoids, however, bind to different receptors not included in the endocannabinoid system (e.g. thermo-TRPs (transient receptor potential channels)).

Phytocannabinoids tend to be extremely lipophilic [60]. The most researched phytocannabinoids are THC and CBD [55,56]. One of the main reasons for this is the fact that they are the main components of resinous cannabis extracts, with CBD accounting for up to 40 % [61] of

the extract and THC accounting for up to 15 % of the dry plant mass [62] (in drug type variants). In addition to the high lipophilicity, some cannabinoids, like cannabidiol or CBD [63,64], exhibit relatively low melting points (< 80 °C).

THC (Figure 2—6) is the main phytochemical of cannabis and mainly responsible for the psychoactive effects of the plant and its extracts [65]. THC shows euphoriant, analgesic, anti-inflammatory, antioxidant and antiemetic properties [1]. Due to the importance for this work as the model substance, the physicochemical and pharmacological properties of CBD are discussed in 3.1.1.

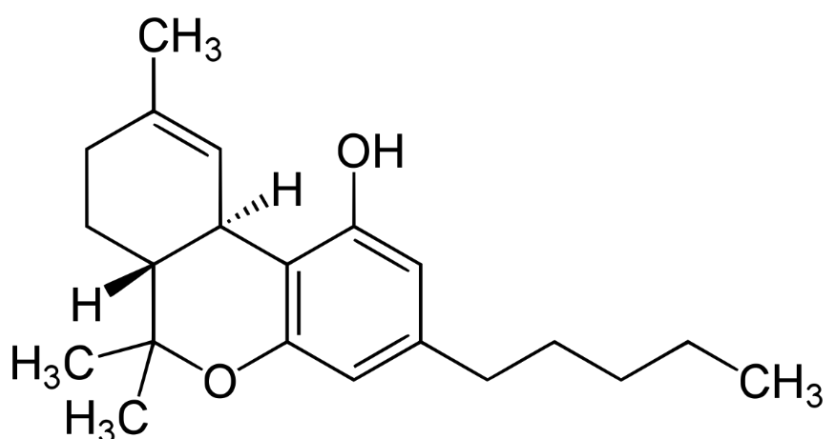


Figure 2—6: Structural formula of THC, adapted from [66]

The first cannabinoid containing pharmaceutical, which was approved in 2005 [67] in Canada and in 2010 in Europe [68] was, Sativex<sup>®</sup> spray. It is a Nabiximols containing oromucosal spray used in the therapy of multiple sclerosis (MS) [67,68]. Nabiximols is a combination of CBD and THC in a 1:1 ratio [67]. Since then cannabis plant material has been approved for medical and, in some states, also for recreational use (also see chapter 1.1). Additionally, Epidiolex<sup>®</sup>, a pure CBD formulation, has been approved as an orphan drug in 2018. It is used in the therapy of the Lennox-Gastaut and Dravet syndroms, two rare forms of infantile epilepsy. On 31 July 2020, the U.S. Food & Drug Administration (FDA) additionally approved the use of Epidiolex<sup>®</sup> in the treatment of seizures associated with the tuberous sclerosis complex [69].

As the most common way of (illicit) application for cannabis has been inhalation, most approaches follow this path. These include (recreational) pMDIs (see chapters 1.2 & 5.1.1), the vaporisation of plant material or oils, or the innovative Syqe<sup>®</sup> inhaler. The Syqe<sup>®</sup> inhaler is a pharmaceutical device (approved in Israel), which contains a cartridge with “VaporChips”. These VaporChips contain a uniform amount of standardised cannabis plant material. The inhaler can vaporise a precise cannabinoid dose utilising electronic dose control [70].

## *2.4 OPTIONS FOR SUBSTANCES WITH FORMULATION DIFFICULTIES*

There are different varieties of formulation difficulties with the most prominent example, which is exhibited by cannabinoids, being physicochemical problems (here: low melting point, crystallinity/amorphicity, lipophilicity). However, a distinct reactivity (i.e. degradation or reaction with excipients) or even a high dose can also be considered formulation difficulties. Substances showing these problems need sophisticated formulation strategies to be made pharmaceutically applicable.

An example for a substance with formulation difficulties due to a high dose is tobramycin (also see 2.2.3). The development of the PulmoSphere™ [53] technology opened the possibility of diverging from the formulation of an interactive blend. An interactive blend does normally not contain more than 4 % [49] of API, which means that a patient would have to inhale 700 mg of formulation to get the declared amount of tobramycin (28 mg [43]). However, it is not known if this, although most of the excipient would deposit in the oropharynx, would result in adverse effects [52]. Adverse effects could, for example, be due to the excipient itself or residual API, which would subsequently be ingested and result in systemic reactions or, in the case of antibiotics, disrupt the intestinal flora. Other problems could be a change in the aerodynamic behaviour of the formulation (e.g. deposition in the inhaler and thus a changing pressure drop) or the triggering of coughing. Additionally, the effects on the lungs are unknown for most excipients. This is especially problematic for formulations that primarily or completely deposit in the lungs, as these subsequently need more extensive toxicological studies.

PulmoSpheres™ are generated through spray-drying from a lipophilic emulsion of perfluorooctyl bromide (Perflubron) in water. The emulsion is stabilised with a long-chain phospholipid (i.e. distearoylphosphatidylcholine), phospholipids are also part of the human surfactant, which signals good tolerability. During spray-drying the water evaporates before the perflubron. This results in the formation of a shell, when the perflubron evaporates pores are formed, leading to the sponge-like structure of the PulmoSpheres™. Since spray-drying being a rapid process, the tobramycin in the PulmoSpheres™ is an amorphous solid [53]. Amorphous substances generally have a higher dissolution rate than crystalline substances [71] and are therefore absorbed faster. The amorphous state is an energetically unfavourable state, which means that substances tend to recrystallise [28]. The amorphous state, however, can be stabilised with the utilisation of a solid dispersion [72].

Substances exhibiting low melting points, a proneness to degradation or a bad aqueous solubility can also be formulated as solid dispersions. A dispersion of a drug in a solid matrix is

defined as a solid dispersion. Today, polymers are mainly used as the matrix, while small molecule matrices have moved to the background [73]. If the drug exhibits a distinct lipophilicity, a solid lipid can be utilized as the matrix. These particles, however, are generally referred to as solid lipid particles [74]. Polymers which have been investigated for solid dispersions include cellulose ethers [75–77], Soluplus® (a polyvinyl caprolactam–polyvinyl acetate–polyethylene glycol graft co-polymer) [78] or polyvinylpyrrolidone [79], the former two were used in this study. The suitability for inhalative purposes has to be evaluated individually. It must be considered that some polymers can be used to produce sustained-release formulations, which might hamper the suitability to produce formulations that require a rapid onset.

Generally spoken, a molecularly dispersed drug in the matrix is the most desirable type of solid dispersion (Figure 2–7A).

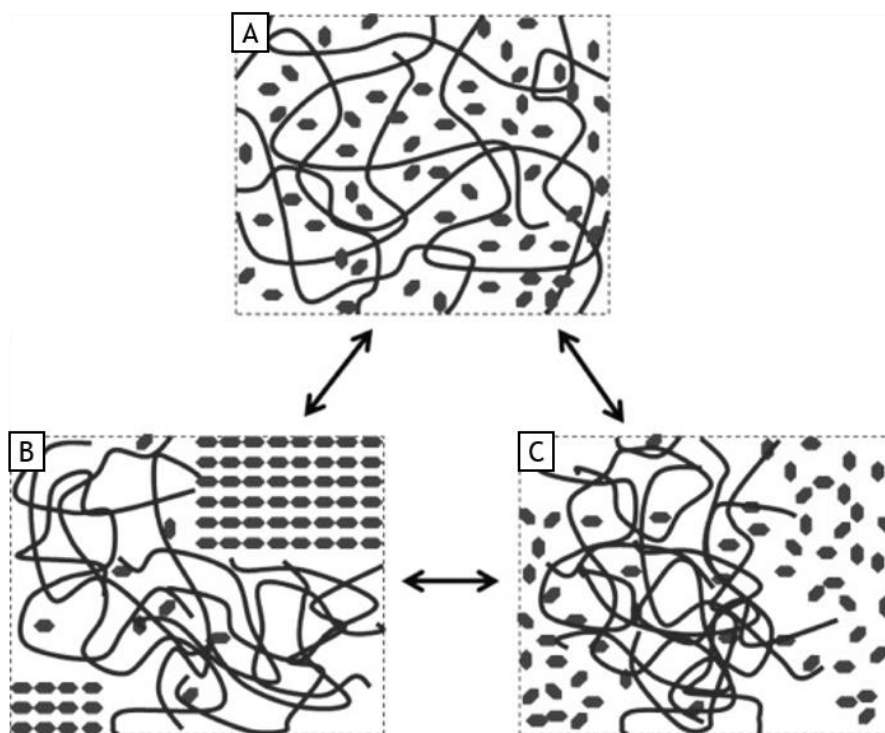


Figure 2–7: The three possible structures of a solid dispersion (lines – matrix, hexagons – drug), molecular dispersion (A), domains with crystalline drug (B), domains with amorphous drug rich areas (C) [73]

Solid dispersions can be prepared by melt extrusion (drugs with low melting point), spray-drying (drugs that are soluble in a volatile solvent) or co-precipitation (drugs with high melting points and low solubility in common organic solvents). Substances, which have been approved as solid dispersion formulations, include itraconazole (Sporanox®, spray-drying & Onmel®, melt extrusion), ivacaftor (Kalydeco®, spray-drying) and vemurafenib (Zelboraf®, co-precipitation) [73].

Ivacaftor has a poor aqueous solubility of < 0.05 µg/mL, a LogP of 5.68 and a melting point of 292 °C, which indicates that a traditional formulation is challenging [80]. The focus of the Kal-ydeco<sup>®</sup> formulation development was the solubility enhancement. The high glass transition temperature ( $T_g$ : 175 °C) of ivacaftor is an indicator of a relatively stable amorphous form; therefore, a spray-dried dispersion was developed. The amorphous form is about 32-fold more bioavailable than the crystalline forms. The developed formulation contains 50 % of ivacaftor in hydroxypropyl methylcellulose and exhibits a 12000-fold better solubility than the crystalline API [80].

Possible advantages of lipid matrices include a controlled drug release and targeting, protection against API degradation, incorporation of both hydrophilic and hydrophobic drugs and they can be produced of physiological and well-tolerated lipids [74]. Glycerol behenate microparticles have already been evaluated as a possible lipid matrix for inhalative purposes (budesonide [81]) and these particles seem to show no significant inflammatory response in rats [74], although more research is needed for long-term application and toxicity [81].

A third approach to formulate these substances is utilised in the Technosphere<sup>®</sup> technology (also see 2.2.3). Here, the drug (insulin) is adsorbed to the surface of a special carrier, which can bind relatively large amounts of insulin. Insulin can be considered a substance with formulation difficulties, as peptides often do not tolerate higher temperatures, organic solvents, or large amounts of salt.

Other substances/products that can be used for this strategy are marketed by Grace. These are synthetic silica products (e.g. SILSOL<sup>®</sup>, SYLOID<sup>®</sup>), which are characterised by a very high porosity and good lipid binding capacities. SILSOL<sup>®</sup> can enhance the bioavailability (binds the drug in an amorphous form) and increase the stability (recrystallisation can be prevented) [82]. SYLOID<sup>®</sup> has a high binding capacity for oils and can thereby transform liquids into solids while also enhancing the bioavailability and API loading and still maintain a quick release of the drug [82,83]. A general advantage of silica is its inertness (listed in FDAs inactive ingredient database). A question mark regarding pulmonary application for silica, however, is the potential toxicity, as chronic inhalation of silica can lead to silicosis and ultimately to lung fibrosis [84].

A further approach is the complexation of the API with cyclodextrins (CDs). CDs are cyclical oligosaccharides, with a lipophilic cavity, consisting of  $\alpha$ -glucose. The most prominent unaltered CDs are  $\alpha$ -,  $\beta$ - and  $\gamma$ -cyclodextrin consisting of six, seven and eight (see Figure 2–8), respectively, glucose monomers. Natural CDs exhibit a somewhat limited aqueous solubility, although glucose is a hydrophilic molecule, thus modified CDs were developed (e.g. 2-hydroxypropyl- $\beta$ -cyclodextrin) which are preferred for aqueous pharmaceutical solutions [85].

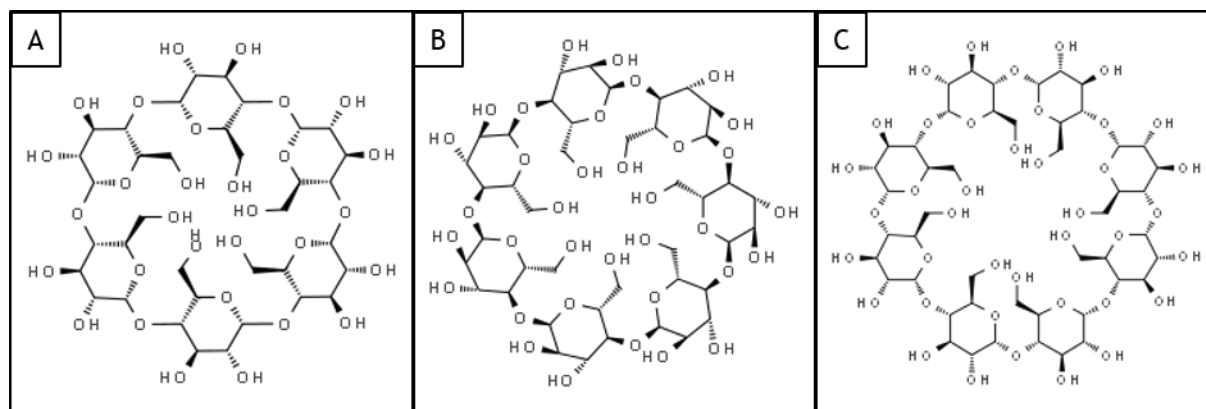


Figure 2-8: Structural formulas of  $\alpha$ -cyclodextrin (A) [86],  $\beta$ -cyclodextrin (B) [87],  $\gamma$ -cyclodextrin (C) [88]

CDs are commonly referred to as enabling pharmaceutical excipients or host molecules. They are able to form inclusion complexes with drugs (guest molecules) by taking a part of the molecule into the central CD cavity and thus change its physicochemical properties [85]. In solution these complexes are in a dynamic equilibrium with free drug molecules and (normally) not covalent [89]. Most drugs will form a complex with a CD-drug ratio of 1:1, although other ratios are possible. The complex formation is dependent on the size of the CDs cavity. For most drugs  $\beta$ -cyclodextrin has the best suitable cavity [85].

The formulation of Cannabinoids has been researched utilising some of the aforementioned technologies, especially the formulation of solid dispersions (THC in inulin [90,91]), solid lipid particles (CBD in Labrafac<sup>®</sup> lipophile WL 1349 (medium-chain triglycerides of caprylic and capric acid triglycerides) [92]) and cyclodextrin inclusion complexes (CBD in  $\beta$ -CD [93]). This work is focussed on the development of an inhalable solid dispersion of CBD, as the production of solid dispersions can be easily combined with production techniques that yield particles in an inhalable size range. However, particles in that size range often exhibit exceptional interparticulate cohesion which can lead to performance related problems or difficulties during filling procedures.

### 3 MATERIALS

#### 3.1 SUBSTANCES

##### 3.1.1 CANNABIDIOL

Today, it is a matter of debate if the different cannabis varieties are subspecies from cannabis sativa or different species. This is because cannabis plants readily interbreed [94], which results in varying fractions of the secondary plant metabolites. In Germany medical cannabis can be obtained as an extract, as a granulate or as flowers from several companies. Table 3—1 shows the three available types of medical cannabis on the basis of the Cannamedical® portfolio: high THC (here: THC: 13.5 – 24.0 %; CBD: 1.0 %), balanced (here: THC: 6.0 – 10.0 %; CBD: 6.0-10.0 %) and high CBD (here: THC: 1.0 %; CBD: 10.0 %). These products are labelled to originate from cannabis sativa, cannabis indica or hybrid variants [93].

Table 3—1: Different types of medical cannabis on the basis of the Cannamedical® portfolio [94]

<i>Product</i>	<i>THC [%]</i>	<i>CBD [%]</i>
<i>Cannamedical® Sativa/Indica/Hybrid forte</i>	24.0	1.0
<i>Cannamedical® Sativa/Indica/Hybrid</i>	20.0	1.0
<i>Cannamedical® Sativa/Indica/Hybrid light</i>	16.5	1.0
<i>Cannamedical® Sativa/Indica/Hybrid vita</i>	13.5	1.0
<i>Cannamedical® CBD forte</i>	10.0	10.0
<i>Cannamedical® CBD</i>	8.0	8.0
<i>Cannamedical® CBD light</i>	6.0	6.0
<i>Cannamedical® CBD vita</i>	1.0	10.0

CBD (Figure 3—1) is the most prominent cannabinoid found in fiber-type cannabis [1]. As stated in chapter 2.3, it also is a major part of the cannabis sativa plant extract (up to 40 %). Concentrated CBD extracts (e.g. the extract used in the stability study) are brownish and resin-like. Due to the reasons stated in chapter 1.2 it was chosen as the model substance for this work.

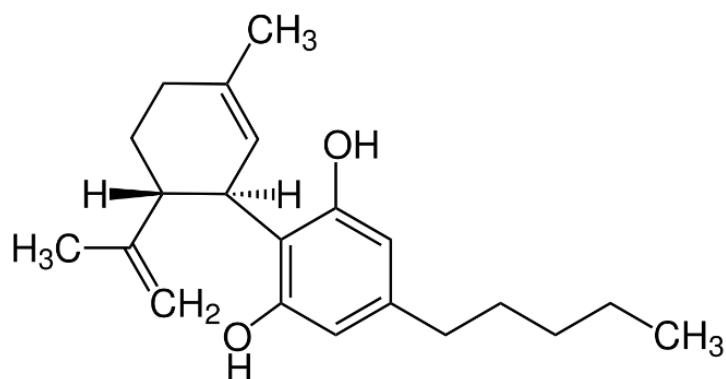


Figure 3–1: Structural formula of Cannabidiol, adapted from [64]

CBD was isolated in 1940 and the structure was first elucidated by Mechoulam and Shvo in 1963 [1]. It is a photoreactive substance and can be oxidised easily, which leads to monomeric and dimeric hydroxyquinones. In the presence of acids CBD can cyclise, yielding THC and iso-THC, while reactions under basic conditions lead to an isomerisation to  $\Delta^6$ -CBD [8]. It can be synthesised via several reactions and the metabolism is well known, as several metabolites have been identified [8].

Pharmacologically, CBD has been attributed a plethora of effects. These include anticonvulsive, sedative (even hypnotic), anxiolytic, antipsychotic, anti-inflammatory and anti-nausea effects [95]. Newer publications also investigate a potential role of CBD in pain management [67]. In contrast to THC, CBD does not have any psychoactive properties.

CBD shows a very low affinity to CB<sub>1/2</sub> receptors (also see 2.3); it rather exhibits antagonistic effects to agonists of these receptors [96]. The mechanisms of action have not been fully discovered, which, again, is a difference to THC. Some postulated mechanisms include the inhibition of anandamide uptake and its antioxidative properties [95,96]. Mechoulam et al. published a review in 2007 [96], in which they present some mechanisms of action for CBD (e.g. action on the serotonin (5-HT) 1A receptor or enhancement of adenosine signalling) as well as some selected biological effects (e.g. allosteric modulation of opioid receptors or CBDs effects on sleep).

As the medical use of CBD is a relatively new field, daily doses have only been established for the treatment of seizure disorders (2400 mg) and psychosis (800 mg). The exact doses for other effects or indications are mostly unknown. Several patients, however, report that daily doses of 5 – 20 mg/day, administered orally in two to three doses, are sufficient to obtain benefits in other indications [97]. CBD shows a relatively poor oral bioavailability of about 16 %, whereas the inhaled bioavailability is reported to be about twice as high with a mean value of 31 % [61].



Based on these values it can be hypothesised that 2.5 – 10 mg/day of CBD would have to be inhaled per day, spread over two to three separate doses. Therefore, each inhalation should administer 0.83 – 3.33 mg of CBD to the lungs.

CBD, both pure (Canapure<sup>®</sup>, Symrise AG) or in a concentrated extract (94 %, Aifame GmbH), is a highly crystalline (Figure 10–1 & Figure 10–2) and extremely lipophilic substance with a logP value (for the pure variant) between 5.79 and 6.92 (depending on the used analytical method) [60]. This is also suggested by DVS analysis, as the maximum uptake of water is not determinable, due to the noise and measuring artefacts being higher than the result, which is below 0.05 % total water uptake. However, DVS measurements are not suitable to determine absolute lipophilicity, although very lipophilic substances tend to show very low water uptakes. DSC analysis of the used substance showed a melting point of 70.14 °C, which is slightly higher than the values (62–63 °C, 66 °C) reported by other sources [64]. The melting point of the used CBD extract (94 %) is slightly lower at about 58 °C. DSC analyses also showed that CBD does not recrystallize during cooling; neither the cooling nor the second heating curve showed any thermic events. HyperDSC<sup>®</sup> analyses revealed that the  $T_g$  of CBD is about 3 °C (Figure 10–3).

### 3.1.2 MATRIX POLYMERS

This study centres around the formulation of solid dispersions of CBD for pulmonary application. Three different polymers were chosen as possible matrix substances for this task.

#### 3.1.2.1 Hydroxypropyl Methylcellulose

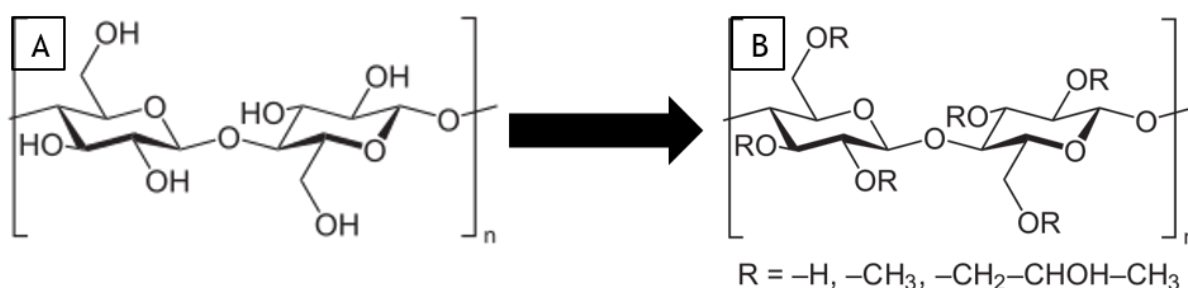


Figure 3–2: Structural formulas of cellulose (A) and hydroxypropyl methylcellulose (B)

Hydroxypropyl methylcellulose (Hypromellose, HPMC, Figure 3–2B) is a semi-synthetic derivative of cellulose (Figure 3–2A). This means that the basic structure of this polymer is a long chain of  $\beta(1 \rightarrow 4)$ -glycosidic linked D-glucose monomers. To convert the hydroxyl groups into methoxy and hydroxypropoxy groups, cellulose is processed with caustic soda before it reacts with methyl chloride and propylene oxide [98].

HPMC comes in different types with varying properties. The properties can be controlled by different degrees of polymerisation (viscosity of a solution), varying percentages of methoxy and hydroxypropoxy groups (solubility) and degrees of substitution (here: resistance against enzymatic degradation) [99,100]. Different properties would yield different products. A higher solubility in organic solvents would for example result in smaller particles, whereas a high viscosity could hamper the processibility, as the peristaltic pump of the Büchi B-290 can only convey solutions up to a viscosity of 300 mPas.

HPMC shows good water solubility and some varieties can be dissolved in organic solvent/water systems. An aqueous solution of HPMC (Methocel products) has an increased viscosity (depending on the degree of polymerisation) and will gel when heated above a particular temperature (thermal gelation) but go back into solution upon cooling. It also shows surface activity, meaning it can be used to stabilise emulsions or suspensions. Due to their high degree of substitution, it is relatively resistant against enzymatic degradation. Another property is a very high pH-stability (2.0 – 13.0). HPMC can also be used to form clear, flexible films or as a binder, which is very interesting for tableting [99].

Different varieties of HPMC have already been used as a matrix in solid dispersions [75–77].

For this work Methocel™ E5 Premium LV (Colorcon GmbH, Idstein, Germany) was chosen. This HPMC variety shows a relatively low viscosity (of a 2 % aqueous solution) of 4 – 6 mPas and has a methoxyl content of 28 – 30 % and a Hydroxypropyl content of 7 – 12 %. Another prerequisite for the planned formulation is the relatively good solubility in EtOH:water mixtures [100].

### 3.1.2.2 Hydroxypropyl Methylcellulose Acetate Succinate

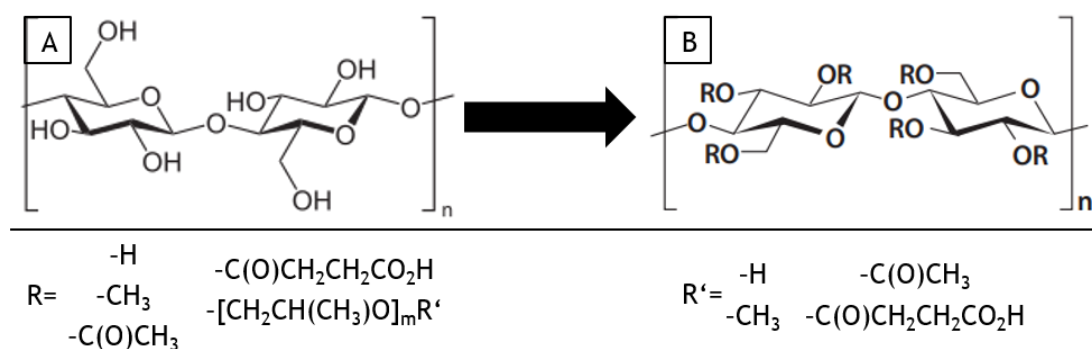


Figure 3–3: Structural formulas of cellulose (A) and hydroxypropyl methylcellulose acetate succinate (B)

Hydroxypropyl methylcellulose acetate succinate (Hypromellose acetate succinate, HPMC-AS, Figure 3–3B) is also a semi-synthetic derivative of cellulose (Figure 3–3A). Here, two

further substituents are introduced: acetyl and succinoyl groups. These groups are added by having HPMC react with acetic anhydride and succinic anhydride [101].

As with HPMC, HPMC-AS can be tailored to exhibit different properties by altering the percentage share of the substituents. For example, Ashland Inc. is offering three different types of HPMC-AS (Table 3–2). All three variants are insoluble in acidic aqueous solutions but soluble in dilute caustic solutions and to various degrees in acetone and MeOH. They also exhibit a very low viscosity in solution, which is advantageous for spray-drying, and a  $T_g$  of about 120 °C [101].

*Table 3–2: Composition of different HPMC-AS types (AquaSolve™), adapted from [101]*

<i>Property</i>	<i>L-Type</i>	<i>M-Type</i>	<i>H-Type</i>
<i>Acetyl content [%]</i>	5 – 9	7 – 11	10 – 14
<i>Succinoyl content [%]</i>	14 – 18	10 – 14	4 – 8
<i>Methoxyl content [%]</i>	20 – 24	21 – 25	22 – 26
<i>Hydroxypropoxy content [%]</i>	5 – 9	5 – 9	6 – 10
<i>Viscosity of a 2% solution at 20 °C [mPas]</i>	2.4 – 3.6		

The polymer has been used as an enteric film-coating for tablets and capsules. However, its effectiveness as a solid-dispersion carrier has attracted the most attention, as numerous publications have shown that HPMC-AS can maintain supersaturation for a wide variety of structures. The acetyl and succinoyl substitution levels have a significant impact on the performance of HPMC-AS as a matrix for solid dispersions [101].

HPMC-AS is incompatible with strong acids or bases as well as with oxidising agents and sustained levels of high humidity. The aqueous insolubility and high molecular weight range (10000 – 500000 dalton) indicate a negligent oral bioavailability, and several toxicological studies showed no adverse effects [101].

AquaSolve™ types LF and MF were chosen for this work. The F in the typification indicates a fine particle size, which ensures a rapid dissolution rate.

### 3.1.2.3 Soluplus®

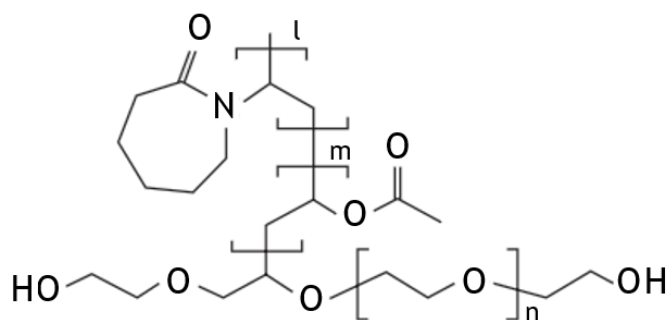


Figure 3—4: Structural formula of Soluplus® [78]; l = vinylcaprolactam; m = vinylacetate; n = PEG

Soluplus® (Figure 3—4) is a polymer which has been developed by BASF SE (Ludwigshafen, Germany) for the formulation of solid solutions. Due to its amphiphilic and bifunctional structure it can be used both as a matrix for solid solutions and as a solubiliser for poorly soluble drugs in aqueous media, thus increasing the bioavailability [78].

Polyethylene glycol (PEG) makes up 13 % of the polymer, while vinylcaprolactam makes up 57 % and vinyl acetate makes up 30 %. The average molecular weight of Soluplus® is 118000 g/mol. Its critical micelle concentration is 7.6 mg/L and the micelles are normally between 70 – 100 nm in diameter. Another property is the comparatively low  $T_g$  of about 70 °C. Soluplus® is soluble in water as well as MeOH (up to 45 %) and EtOH (up to 25 %) among others. High polymer concentrations will result in turbid aqueous solutions due to the formation of colloidal micelles. Like other polymers, Soluplus® will increase the viscosity of a solution, albeit not very much below a concentration of 10 % [78].

Soluplus® can be processed with hot melt extrusion and spray-drying to generate solid dispersions [78].

As Soluplus® is not used in a marketed pharmaceutical, as of this point, there is only little information about the long-term toxicity of this polymer. However, Andrade et al. showed that Soluplus® does not exhibit significant in-vitro toxicity on respiratory cell lines [102], which is a main reason for the selection of Soluplus® as the third matrix substance for this study.

### 3.1.3 ANTIOXIDANTS

Antioxidants (AOs) are substances that can be added to formulations to prevent the oxidative degradation of one or more components. AOs can be divided into three subcategories:

1. True antioxidants are believed to block chain reactions by reacting with free radicals. The agent donates electrons and hydrogen atoms which are accepted by free radicals. Examples are tocopherol or butylated hydroxytoluene.

2. Reducing agents have a lower redox potential than the substance they are protecting. This means that they are oxidised more easily than the API. Examples are sodium metabisulfite and ascorbic acid.
3. Synergists enhance the effects of AOs. This can happen by binding copper or iron ions, which catalyse oxidative reactions. Examples are complexing agents like disodium edetate or citric acid [103].

### 3.1.3.1 Ascorbic Acid

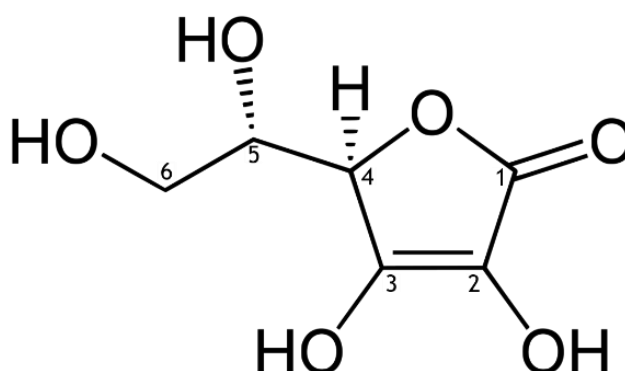


Figure 3–5: Structural formula of ascorbic acid, adapted from [104]

Ascorbic acid (AA), better known as vitamin C, is a commonly used antioxidant with a molecular weight of 176.12 g/mol. The strong reducing power of the molecule is linked to the enediol group, the acidic character results from the hydroxy group at C3. AA is a highly hydrophilic molecule with a LogP value of -0.178 [104]. Although crystalline AA is stable against atmospheric oxygen, it will be degraded quickly by oxidising agents in solution [9]. When AA is oxidised, a yellow discolouration takes place [103].

AA is a substance listed in the GRAS (Generally Recognized As Safe) database of the FDA and carries an SCOGS (Selected Committee on Generally Recognized as Safe Substances) conclusion type of 1. This means that no evidence was found that demonstrates or suggests reasonable grounds to suspect a hazard to the public at currently used levels or levels that can be reasonably expected in the future.

It is mostly used in dietary supplements or food (E300). An example of a pharmaceutical, where AA is added for its antioxidative properties, is Aspirin® Plus C (Bayer Group, Leverkusen, Germany). In this formulation it is added because it showed a positive effect on the human leucocyte immune response (ex vivo and in vitro). Additionally, some studies suggest that it exhibits protective effects the stomach mucosa from acetylsalicylic acid-induced stomach lesions and oxidative stress [105].

As AA has been used to successfully stabilise cannabinoids in whole blood samples [106] it was chosen as one of the antioxidants for this study.

### 3.1.3.2 Ascorbyl Palmitate

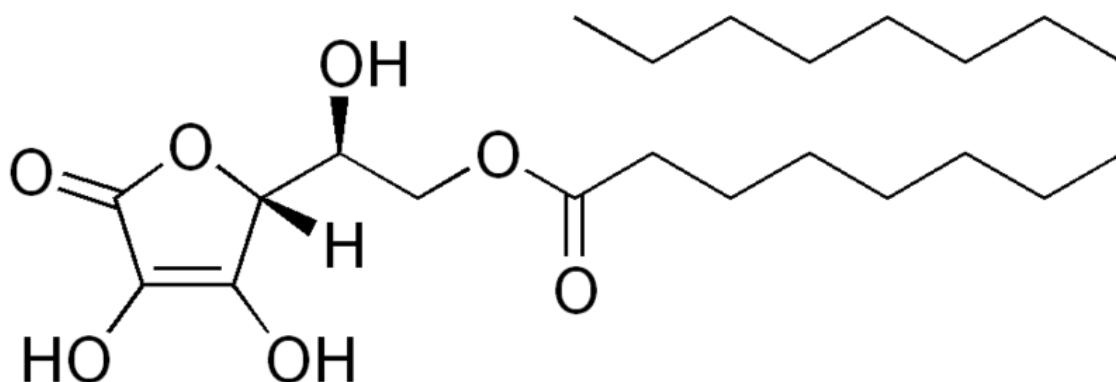


Figure 3–6: Structural formula of ascorbyl palmitate, adapted from [107]

Ascorbyl palmitate (AP) is an ester of AA and palmitic acid. This esterification creates a fat-soluble form of AA. AP has a molecular mass of 414.54 g/mol and the LogP-value of 8.21 [107] signals extreme lipophilicity. AP is used in food (E304) and there is some research regarding its use in topical pharmaceuticals. Additionally it is used as an antioxidant for dronabinol in the German *Neues Rezeptur Formularium (NRF)*, monograph 22.8 [108]).

Like AA, AP is a substance on the GRAS list with an SCOGS conclusion type of 1. Due to its higher lipophilicity, compared to AA, and its use with a related substance, AP was chosen as one of the AOs for this study.

### 3.1.3.3 Butylated Hydroxyanisole

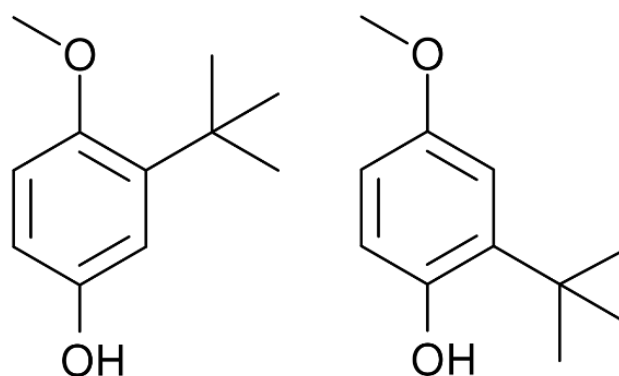


Figure 3–7: Structural formula of butylated hydroxyanisole, adapted from [109]

Butylated hydroxyanisole (BHA) is an AO with a molecular mass of 360.23 g/mol. BHA is a lipophilic molecule that is freely soluble in ethanol. The antioxidant reaction mechanism of

this molecule can follow several paths with the end products being different dimeric structures with orange colour or small aliphatic molecules like hydroquinone [110].

BHA is commonly used as an antioxidant in food (E320) and some pharmaceuticals, most notably with isotretinoin [111], lovastatin [112] and simvastatin [113].

BHA was chosen for this work as it is a common lipophilic AO, which is already used in some marketed products. It is a GRAS substance with an SCOGS type 3 conclusion. This means that no available information evidences a hazard to the public in currently used levels. However, there are uncertainties that require further evaluation.

#### 3.1.3.4 Butylated Hydroxytoluene

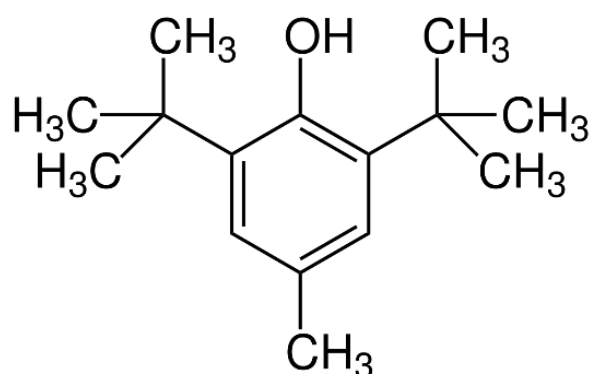


Figure 3–8: Structural formula of butylated hydroxytoluene, adapted from [114]

Butylated hydroxytoluene (BHT) is another very common lipophilic AO (logP: 4.46 [114]) with a molecular weight of 220.35 g/mol. It is manufactured industrially in very high quantities. However, some variants of freshwater phytoplankton can also produce natural BHT [115].

As stated in Chapter 3.1.3, BHT functions as a scavenger with several different end products, depending on the intercepted radical [116].

BHT is commonly used in the fuel industry as well as in food (E321) and pharmaceuticals or cosmetics. Pharmaceuticals containing BHT are mainly topical formulations with APIs like mometasone furoate [117]. The *NRF* uses BHT for lidocaine eardrops (monograph 16.5).

Although there is some concerning data which links BHT to the promotion of lung tumours in mice [118], it was chosen to complete the set of AOs used in this study. Like BHA, it also carries an SCOGS type 3 conclusion.

Further materials and chemicals for analyses are listed in the appendix (chapter 10.3).

## 3.2 INHALATION DEVICES

### 3.2.1 CYCLOHALER®

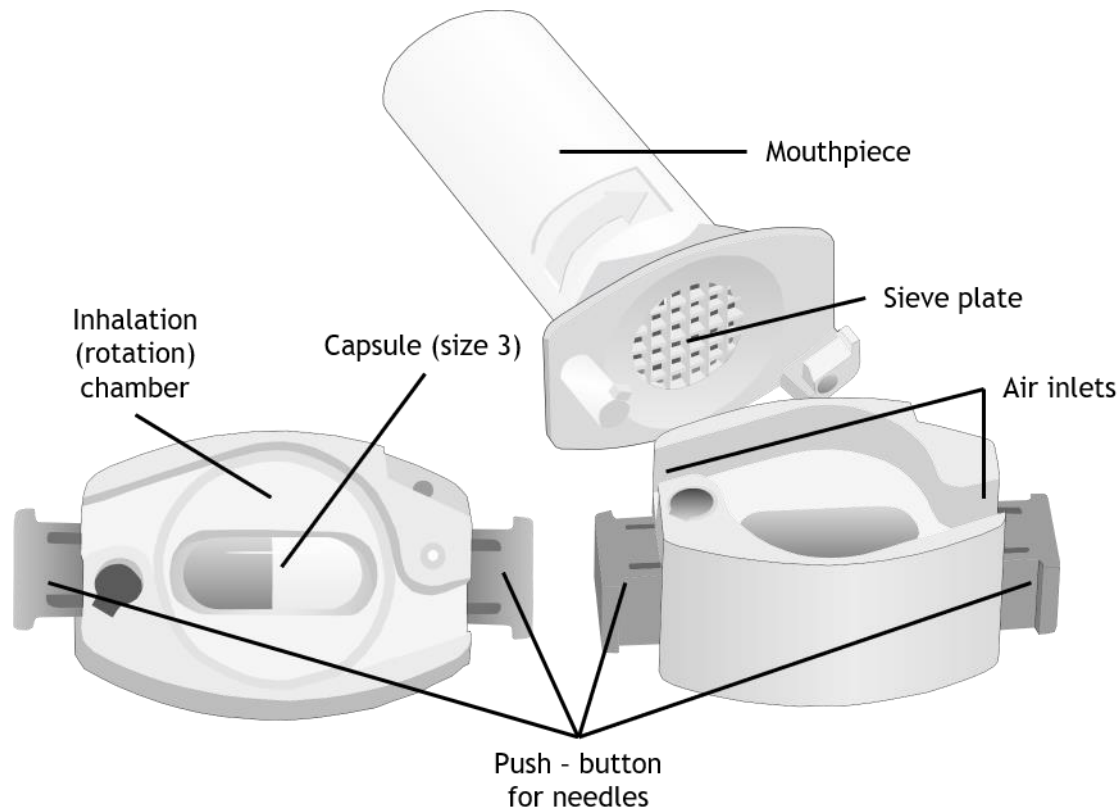


Figure 3–9: Schematic diagram of the Cyclohaler® DPI, left: top view on inhalation chamber, right: disassembled device

The Cyclohaler® (PB Pharma GmbH, Meerbusch, Germany) is a capsule-based, single-dosed DPI, in which the inhalation powder dose is contained in the capsule.

To perform an inhalation manoeuvre, the device must be opened, by twisting the mouthpiece, to insert the capsule (size 3) into the device. The capsule is subsequently pierced by simultaneously pushing the buttons at both sides of the inhaler, which operate four needles each. The puncture of the capsule concludes the preparation of the device for the inhalation manoeuvre.

The capsule will spin rapidly in the inhalation chamber when an air stream is applied through the mouthpiece. This creates relatively strong centrifugal forces which carry the formulation out of the capsule. After leaving the capsule, the powder enters the turbulent airstream, which may lead to collisions with the sieve plate (main task: inhibit inhalation of the capsule). The airstream, the centrifugal forces, and the collisions with the sieve plate lead to the deagglomeration of the powder. After passing the sieve plate, the air stream is decelerated and becomes laminar, resulting in weaker dispersion forces [119].



With the Cyclohaler<sup>®</sup>, beclometasone dipropionate, budesonide and salbutamol sulphate are marketed as Cyclocaps<sup>®</sup> in Germany [120].

### 3.2.1.1 Capsules

For this study HPMC capsules (Capsugel, Morristown, NJ, USA) in size 3 were used, as they are resistant to stringent heat and humidity conditions while also being suitable for moisture sensitive ingredients as well as low-moisture contents [121]. Additionally, they are also acceptable for vegetarians or people who reject gelatine for religious reasons. Each capsule was filled manually to a filling weight of  $15 \pm 0.5$  mg.

### 3.2.2 DISKUS<sup>®</sup>

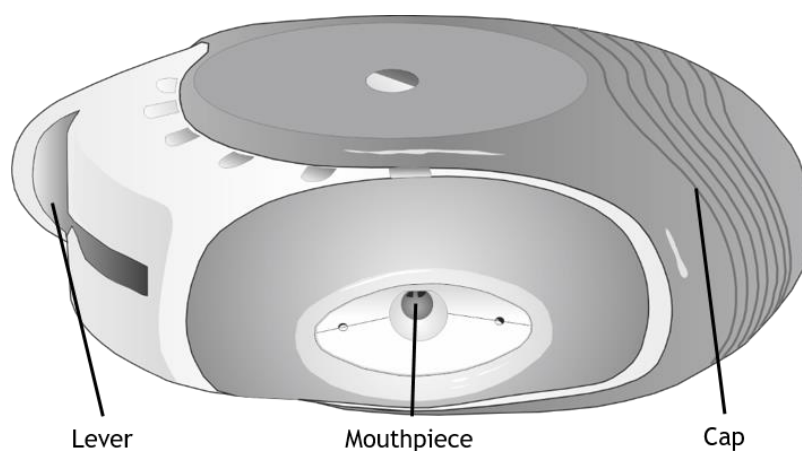


Figure 3–10: Schematic diagram of the Diskus<sup>®</sup> multi-dose inhaler

The Diskus<sup>®</sup> (GlaxoSmithKline GmbH & Co. KG, Munich, Germany) was one of the first available multi-dose DPIs. The first product marketed with the Diskus<sup>®</sup> was Flutide<sup>®</sup> (fluticasone propionate), which was approved in Germany in 1994 [122]. Today, three further products utilising the Diskus<sup>®</sup> are marketed in Germany: atmadisc<sup>®</sup> (fluticasone propionate & salmeterol xinafoate), Serevent<sup>®</sup> (salmeterol xinafoate) and Viani<sup>®</sup> (salmeterol xinafoate & fluticasone propionate). Each product contains a blister with 60 doses per device.

To prepare the device for inhalation, the cap is rotated until it latches on the other side. Now the lever can be pushed from the mouthpiece towards the counter. This moves gears inside the inhaler, which opens one blister cup and moves it behind the mouthpiece, while moving down one dose on the counter. After the inhalation manoeuvre the cap is rotated back in front of the mouthpiece, this also causes the lever to snap back into its original position. The device has no deagglomeration enhancing features apart from a cross inside the short mouthpiece.

The Diskus<sup>®</sup> was chosen for this study, because it is an example for pre-metered multiple-dose inhalers. Additionally, it is relatively easy to take the device apart without damaging any

parts. This enables the installation of an alternative blister strip. Furthermore, it was possible to fill appropriate blisters (see 3.2.2.1).

### 3.2.2.1 Blister Strips

For the aluminium-aluminium blister strips, two different foils were used [123,124]. Figure 3–11 shows a blister from the top, side and bottom.

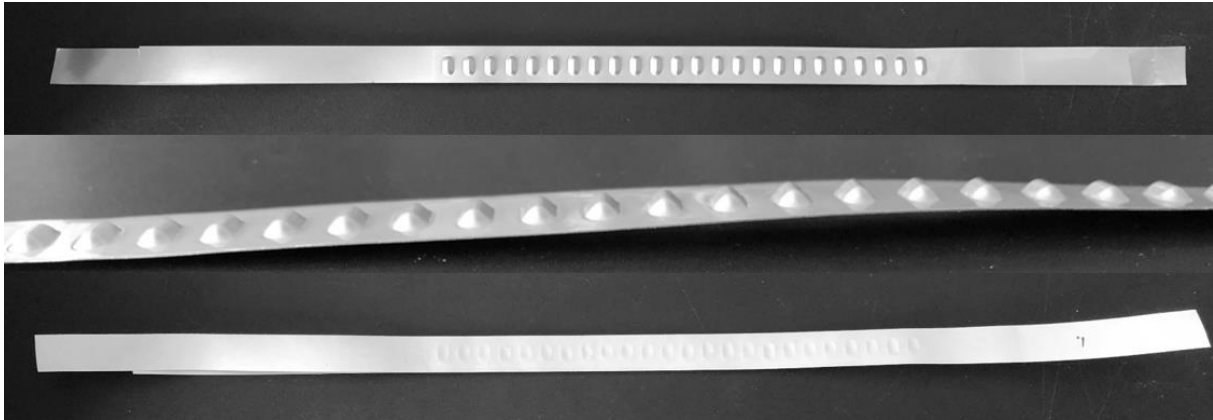


Figure 3–11: Blister for the Diskus® device; top: top view; middle: lateral view; bottom: bottom view

Two rows with 24 cups each (volume: 18 – 20 mm<sup>3</sup>) were thermoformed into the bottom foil. Then the powder was volumetrically filled into the cups with a membrane filling machine (Figure 3–12, Harro Höfliger Verpackungsmaschinen GmbH, Allmersbach im Tal, Germany) under standardised conditions (20 °C, 50 % relative humidity (rH)). To fill the cups efficiently, the vacuum (-600 mbar for 400 ms) and the fluidisation flow (300 mbar for 100 ms) had to be applied six times while the powder bed was continuously moved with a fitted scraper. This leads to a slight compaction of the powder. The cups were subsequently sealed with the top foil by applying 180 °C for 800 ms. Finally, the two blister rows were cut apart to enable the installation in the Diskus®.

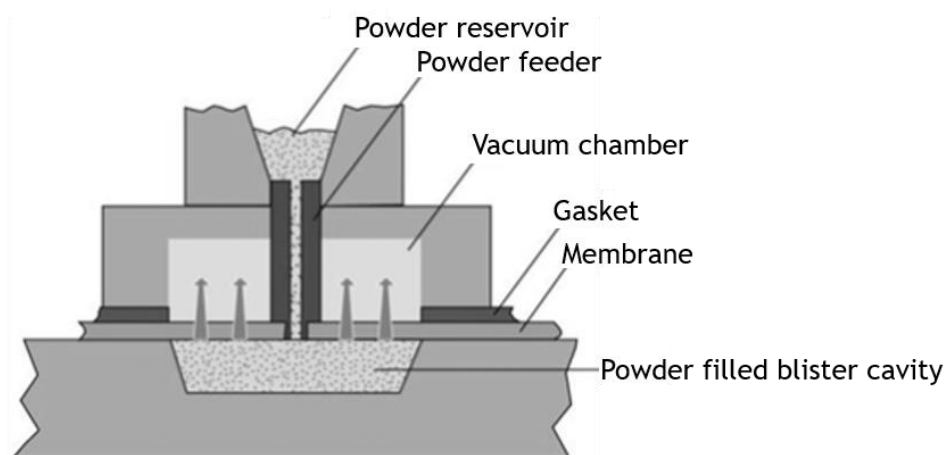


Figure 3–12: Schematic diagram of the used membrane filling machine

The filling of the blisters was carried out at Harro Höfliger Verpackungsmaschinen GmbH (Allmersbach im Tal, Germany).

### 3.2.3 *pMDIs*

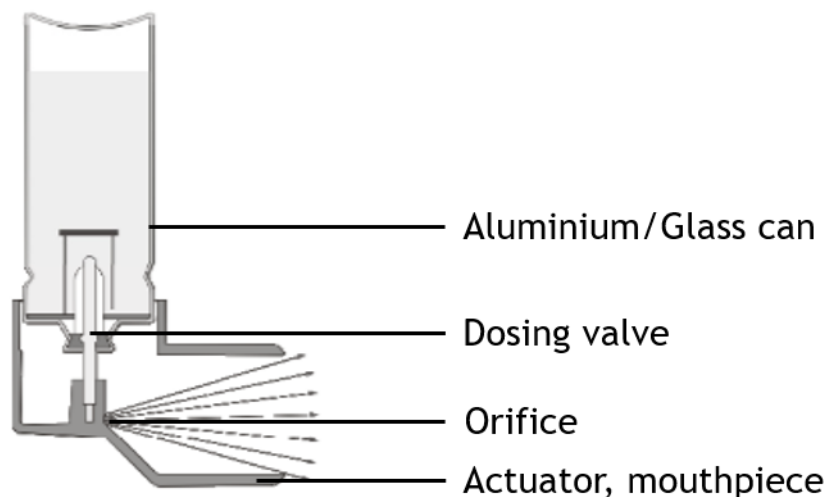


Figure 3–13: Schematic diagram of a conventional *pMDI*

*pMDIs* (Figure 3–13) are simple devices that made up of a can (normally aluminium) with a metering valve (e.g. 100  $\mu\text{L}$ ). These closed containers contain the propellant-containing (e.g. HFA 134a) formulation. The actuation is achieved by pushing the exhaust stem of the valve on an orifice (e.g. 0.22 mm).

Two types of cans, glass and aluminium, were used over the course of this project. The former were used during formulation trials, whereas the latter were utilised during aerodynamic characterisation and the stability study. During the formulation studies, two different HFAs (134a and 227) were evaluated as possible propellants.

During the aerodynamic characterisation and formulation experiments 61  $\mu\text{L}$  dosing valves were used (Aptar DF316/61 RCU CS20 AG), while the *pMDIs* of the stability study were crimped with 50  $\mu\text{L}$  dosing valves (Valois DF 30/50 RCU CS 20 Argent).

## 4 METHODS

### 4.1 PREPARATIVE METHODS

#### 4.1.1 SPRAY DRYING

Spray-drying is a widely used method for pharmaceutical applications [125] and a viable method for preparing solid dispersions (see chapter 2.4). It is a thermally gentle [28] procedure which is a major advantage compared to other ways of generating solid dispersions like hot melt extrusion. Therefore, it was chosen as the method for preparation of the solid dispersions of CBD.

Every spray-drying procedure for this work was carried out with a Mini Spray Dryer B-290 coupled with an Inert Loop B-295 (Figure 4–1; both Büchi Labortechnik AG, Flawil, Switzerland). Nitrogen was used as the drying gas to provide an inert processing atmosphere, as organic solvents were used for the feed solutions.

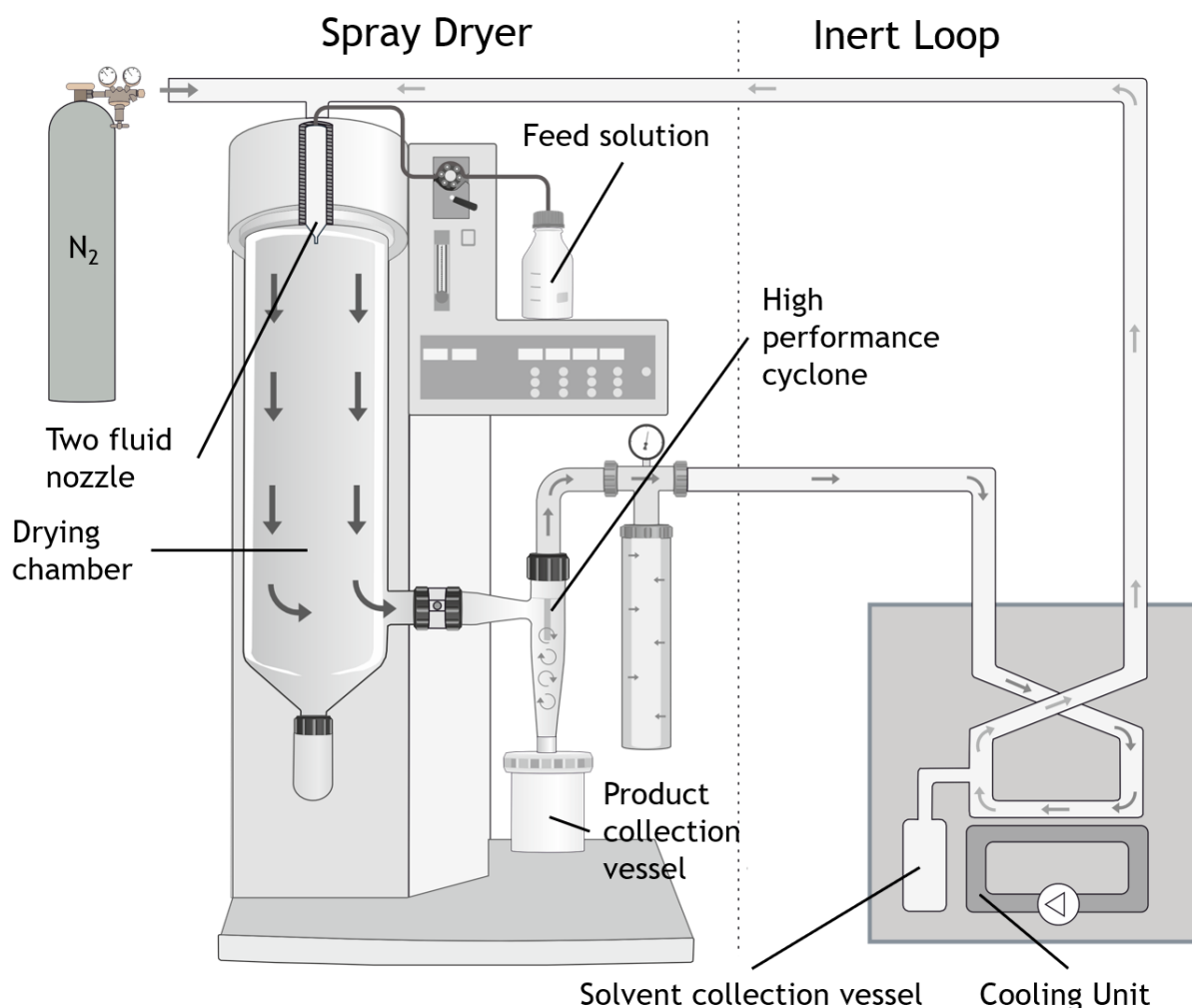


Figure 4–1: Schematic diagram of the Büchi Mini Spray Dryer B-290 (left) with the Inert Loop B-295 (right)

In this case, the process starts with an ethanolic (80 wt%) solution of CBD, the chosen matrix polymer (HPMC, HPMC-AS, Soluplus®) and, if added, the AP. A peristaltic pump feeds the solution to a two-fluid nozzle (inner diameter: 0.7 mm; outer diameter: 1.4 mm), which disperses it into the drying chamber. As soon as the spray-solution droplets contact the hot drying gas, the solvent starts to evaporate. As the amount of solvent reduces, particles (i.e. solid dispersions) are formed. The drying gas then carries the particles to the (in this case: high performance) cyclone, where they are separated from the air flow [125].

The spray-drying parameters for the three different polymers are summarised in Table 4–1. The parameters for HPMC and HPMC-AS were optimised utilising an experimental design (Design of experiments, DoE, also see chapter 4.4.1).

Table 4–1: Used spray-drying parameters for stability samples

<i>Polymer</i>	<i>Inlet temperature [°C]</i>	<i>Aspirator [m³/h]</i>	<i>Airflow [L/h]</i>	<i>Pump rate [mL/min]</i>	<i>Outlet temperature [°C]</i>
<i>HPMC</i>	100	35	601	9	57
<i>HPMC-AS</i>	70	35	601	3	45
<i>Soluplus®</i>	100	35	601	9	60

The quantitative composition of the spray-drying solutions is depicted in Table 4–2. Each formulation should contain 10 % of CBD and, if added, an equimolar amount of AO (here: AP).

Table 4–2: Quantitative composition of the spray-drying solutions for the stability study samples

<i>Formulation</i>	<i>Matrix</i>	<i>Matrix [g]</i>	<i>CBD [g]</i>	<i>AP [g]</i>	<i>Ethanol (80 wt%) [mL]</i>
<i>1, 3, 5</i>	Soluplus®, HPMC, HPMC-AS	18.0	2.0	0	400
<i>2, 4, 6</i>	Soluplus®, HPMC, HPMC-AS	15.36	2.0	2.64	400

### 4.1.2 PMDI FILLING

CBD or CBD and AP were weighed into an aluminium or glass can. 1 mL of EtOH was added to the can before it was crimped with a metering valve. The cans were put in an ultrasonic bath for 60 seconds to dissolve the solids as completely as possible before adding the propellant by weight with a *Laboranlage Typ P 2016* (Pamasol Willi Mäder AG, Pfäffikon, Switzerland, Figure 4–2).

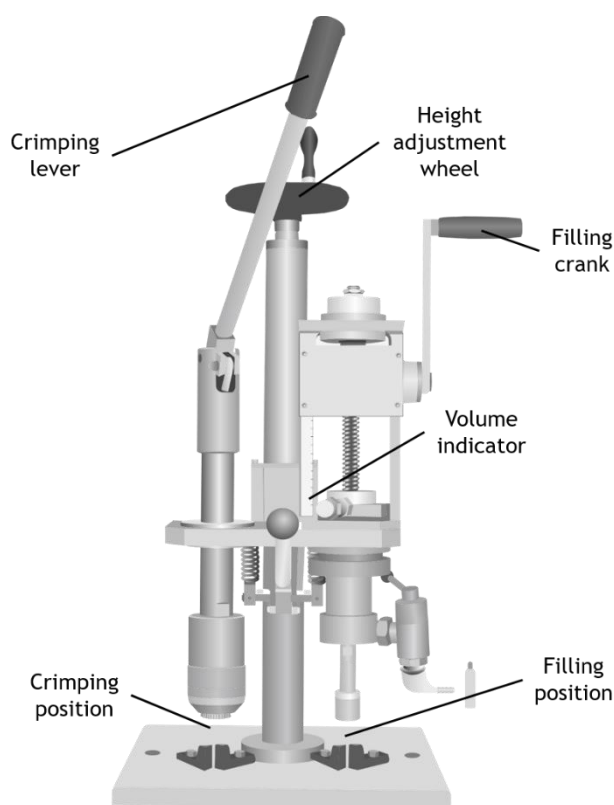


Figure 4–2: Schematic diagram of the used pMDI filling station

The composition of the pMDIs, which were tested for their aerodynamic performance, is depicted in Table 5–1. For this experiment a DoE ( $2^3$  full factorial, see chapter 4.4.1) was implemented.

The quantitative build-up of the pMDIs used for the stability study is depicted in Table 4–3. The formulations have a CBD concentration of 30 mg/mL, as HFA 134a has a liquid density of 1.21 g/mL.

Table 4–3: Quantitative build-up of the stability study samples

Formulation	CBD [mg]	AP [mg]	Ethanol [mL]	HFA 134a [g]
pMDI	360	0	1	13.31
pMDI + AO	360	474.6	1	13.31

## 4.2 ANALYTICAL METHODS

### 4.2.1 LASER DIFFRACTION

Laser light diffraction is a commonly used method to evaluate geometric diameters smaller than 100  $\mu\text{m}$ . Here, the particles are irradiated with a monochromatic light (helium-neon-laser; wavelength: 632.8 nm), which is then diffracted in different intensities at the particles' surfaces. The diffraction angle is inversely proportional to the particle size. This results in large diffraction rings with low intensity for small particles and vice versa for large particles [28]. Diffraction rings of multiple particles will interfere with each other and result in characteristic diffraction patterns. These patterns were evaluated utilising the Fraunhofer-approximation. It must be noted that the Fraunhofer approximation assumes that the measured particles are shaped spherically. This method enables the analysis of particle sizes or rather particle size distributions (PSDs) between 0.1 and 8750  $\mu\text{m}$ .

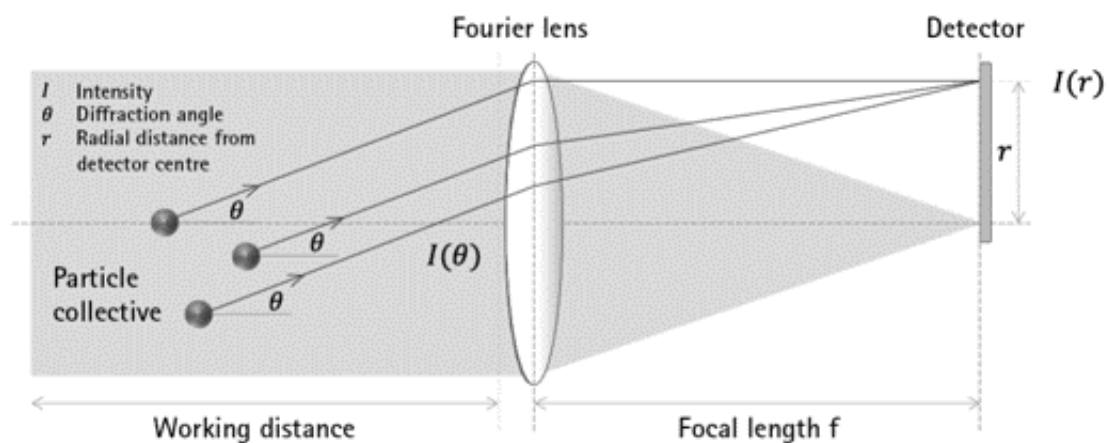


Figure 4–3: Laser diffraction in the optical path of the HELOS [126]

Data was generated with a HELOS laser diffractometer coupled with a RODOS dry dispersion system and a VIBRI dosing unit (Figure 4–4, all: Sympatec GmbH, Clausthal-Zellerfeld, Germany) and analysed with the Windox software (version 5.4.2.0, Sympatec GmbH, Clausthal-Zellerfeld, Germany). The RODOS module disperses the powder with pressurised air. The dispersion pressure must be selected so that a close to optimal dispersion is achieved without destroying the particles. A dispersion pressure of 3 bar was determined to fit the requirements.

Each measurement was preceded by a reference measurement of ten seconds to determine the background. Data was acquired as soon as an optical concentration of 1 % was exceeded and stopped three seconds after falling below 1 %.

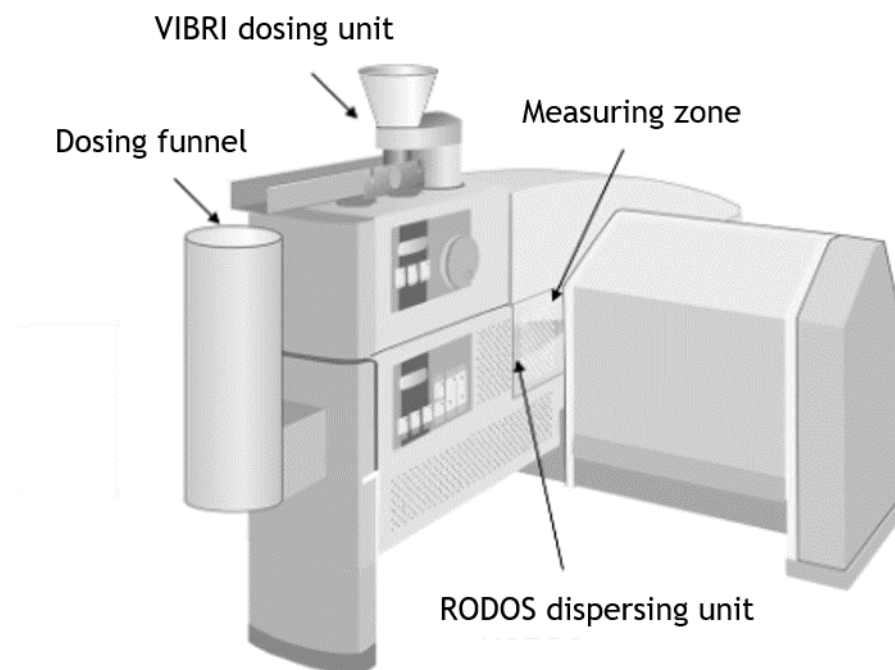


Figure 4–4: Schematic of the measuring setup HELLOS + RODOS + VIBRI

Five lenses were available (Table 4–4). Lens R1 was used after spray-drying the formulations and lens R5 was used after the stability study to account for possible agglomerates.

Table 4–4: Lenses available for the used HELLOS laser diffractometer

<i>Lens</i>	<i>Focal length [mm]</i>	<i>Effective range [μm]</i>
<i>R1</i>	20	0.1 – 35
<i>R2</i>	50	0.25 – 87.5
<i>R3</i>	100	0.5 – 175
<i>R4</i>	200	0.5 – 350
<i>R5</i>	500	0.5 – 875

Measurements were carried out volume based (Q3) and in triplicate and the results were displayed as distribution sum and distribution density curves.

The  $x_{10}$ ,  $x_{50}$  and  $x_{90}$  quantiles as well as the span value are commonly used as characteristic parameters of PSDs. An  $x_{50}$ -value of 100 μm, for example, states that 50 % of the bulk volume (volume based) or 50 % of all measured particles (number based) are smaller than 100 μm. The span value was determined with equation (4–1).

$$\text{span} = \frac{x_{90} - x_{10}}{x_{50}} \quad (4-1)$$



For this work, the  $x_{50}$  was used as the mean particle size while the span was used to determine the width of the PSD.

#### 4.2.2 X-RAY POWDER DIFFRACTION

X-Ray powder diffraction (XRPD) is a method which provides information about the crystal lattice or lack thereof (amorphousness) of a substance or powder blend.

The needed X-Rays are generated by applying up to 50 kV to a cathode (e.g. tungsten), which accelerates the electrons towards the anode. When the electrons hit the anode, both bremsstrahlung and characteristic X-Rays are emitted. The latter is dependent on the anode material and the one used to for XRPD. The sample is placed into the X-Rays path. When the X-Rays hit the sample, the electrons will be diffracted from the electron clouds of the atoms [127] in different angles and intensities. As every crystal modification yields a specific diffractogram, this method can be used for the identification not only of substances but of polymorphs as well.

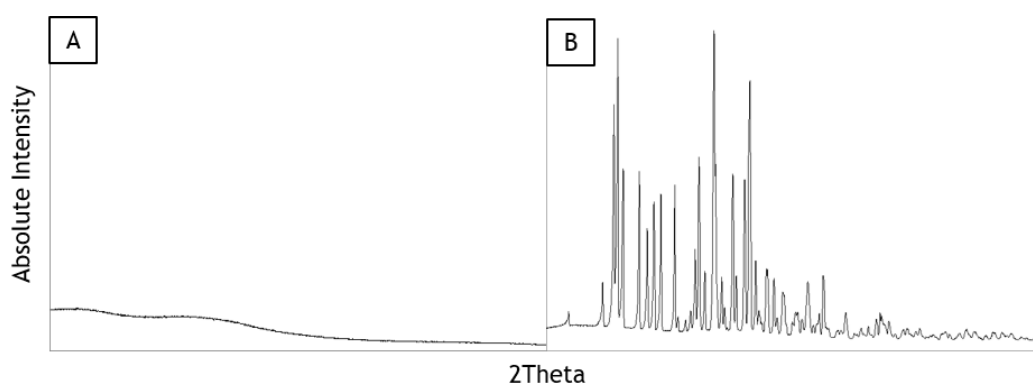


Figure 4–5: exemplary XRPD spectra, fully amorphous “halo” (A), fully crystalline substance (B)

Results are depicted as the intensity plotted against the measuring angle 2Theta ( $2\theta$ ). A fully amorphous sample does not diffract the electrons and can therefore not yield a diffraction pattern. This results in a so-called “halo” (Figure 4–5A). A fully crystalline sample, on the other hand, will diffract the electrons in dependency of its crystal modification, resulting in a distinctive diffraction pattern (Figure 4–5B).

Samples were prepared by placing the powder between two layers of Scotch<sup>®</sup> tape Magic<sup>™</sup> (3M Deutschland GmbH, Neuss, Germany), which is virtually X-Ray amorphous. The diffractograms in this work were taken with a Stadi-Ps (STOE & CIE GmbH, Darmstadt, Germany) in a range of 3 (5) – 50 2Theta in 0.75 2Theta steps with a duration of 60 seconds. The diffraction was measured with a MYTHEN 1K detector (DECTRIS AG, Baden, Switzerland).

Analyses were carried out by Dr. Nicole Pienack at the institute for inorganic chemistry, Kiel University.

### 4.2.3 DIFFERENTIAL SCANNING CALORIMETRY

Differential scanning calorimetry (DSC) is a thermoanalytical method, which can detect multiple physicochemical properties of substances or blends. Key properties, which can be identified with DSC, include the melting point (crystalline substance) and the glass transition temperature (amorphous substance). With these results it is possible to deduce if a sample is crystalline or amorphous.

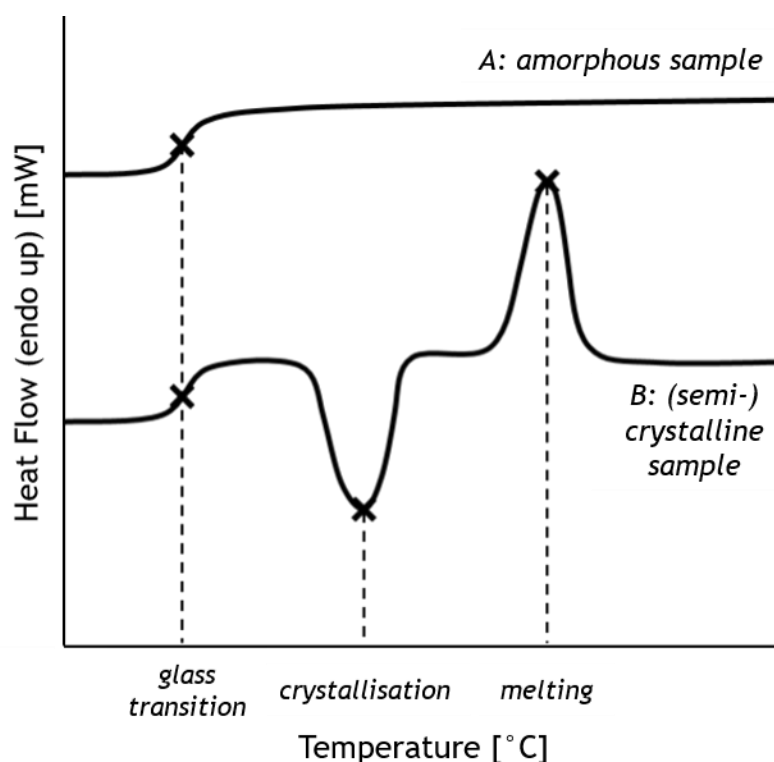


Figure 4–6: Exemplary DSC diagram

Results are normally plotted as the energy flow (mW or J/s) against temperature [28] (Figure 4–6) or time. Changes in the energy flow can be of endo- or exothermic nature. This depends on the phase transition taking place. Melting is an endothermic event while recrystallisation is exothermic, both will normally result in a distinct peak. A glass transition will not result in a distinct peak but rather in a change of the curves' base level. For crystallisation and melting peaks, the onset of a peak was used as the descriptive point, a glass transition is the turning point in the change of the base level.

A power compensation DSC (Figure 4–7) (PerkinElmer PYRIS™ Diamond DSC (PerkinElmer Inc., Waltham, MA, USA)) was used for each measurement during this work and data was evaluated with the PYRIS™ Software (version 9.0.2.0193; PerkinElmer Inc.). 2 – 5 mg of the

respective sample were weighed into an aluminium pan, which was subsequently sealed and pierced, to allow air to escape should the sample expand due to melting. A pierced, empty aluminium pan was used as the reference. During analysis, the sample was flushed with nitrogen gas to avoid oxidation processes.

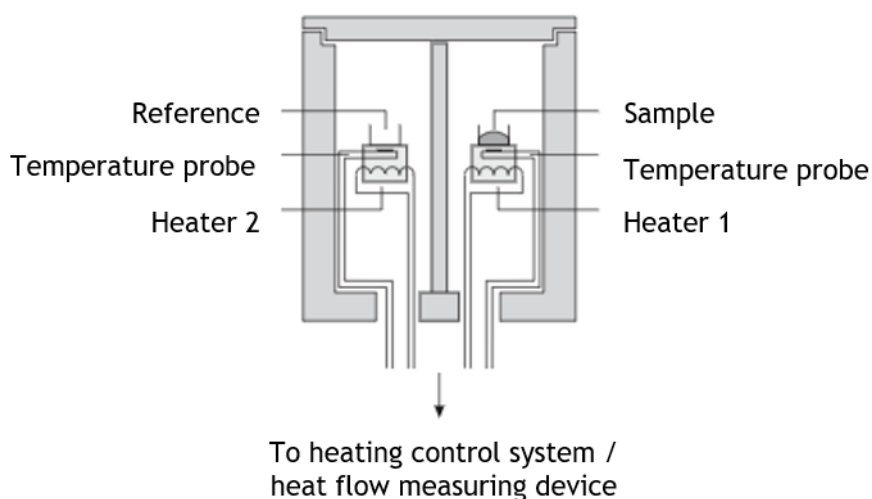


Figure 4–7: Schematic diagram of a power compensation DSC (without flushing), adapted from [128]

Regular samples were heated at a rate of 10 °C/min (to 140 °C) and cooled at a rate of 5 °C/min. Each sample was heated twice to detect possible differences after thermal stress.

For the evaluation of the glass transition temperature the samples were initially cooled to about -80 °C before being heated to 110 °C at different rates (50, 100, 120 °C/min). This is an example of HyperDSC™ [129], which utilises very high heating rates. The increased heating rates increase the sensitivity of the analyses while also enabling a higher throughput.

#### 4.2.4 DYNAMIC VAPOUR SORPTION

Dynamic vapour sorption (DVS) is an incredibly versatile method as it can be used to determine a multitude of properties, i.e. a samples hygroscopicity, its amorphous content, uptake of water or organic solvents and BET-surface. For this work, the usage of DVS was confined to the hygroscopicity and the uptake of solvents. The apparatus itself measures the partial pressure-dependent change in mass of the sample.

A schematic diagram of the DVS Resolution, which was used for this work, (Surface Measurement Systems Ltd., London, UK) is depicted in Figure 4–8. A DVS apparatus consists of a microbalance with a reference and a sample cell, which are flushed with a gas stream (carrier gas & solvent vapour) from the same origin. The adsorption of the vapour to the sample

pan is thereby eliminated and the uptake of the sample can be evaluated. Nitrogen was used as the carrier gas to prevent sample oxidation. The DVS Resolution can evaluate the sorption behaviour of samples to both water and organic solvents. A climate chamber is used to maintain a consistent environment as the partial pressure is dependent on the temperature.

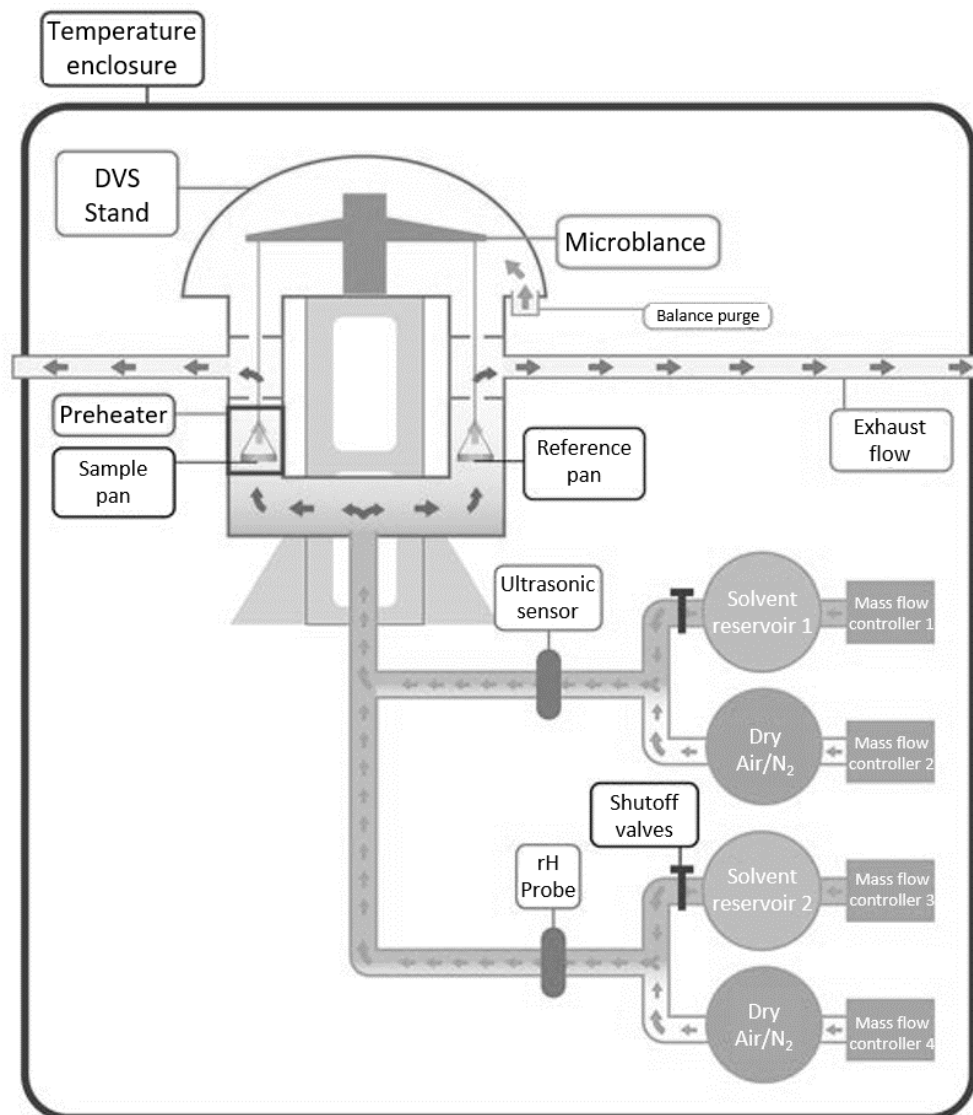


Figure 4–8: Schematic diagram of the DVS Resolution [130]

DVS results are normally plotted as the partial pressure and the sample (change in) mass against time or alternatively partial pressure plotted against (change in) mass.

For this work 5 – 25 mg of the respective sample were weighed into the sample pan. Measurements were carried out at 25 °C. The method consisted of two cycles of increasing the partial pressure (of bidistilled water (aq. bidest.)) from 0 – 90 % in 10 % steps and returning to 0 %. The initial 0 % step was set to 180 minutes while every other step was limited by a change in mass of < 0.005 %/min for a maximum duration of 480 minutes and a minimum duration of 10 minutes.

The hygroscopicity was evaluated according to the thresholds (Table 4–5) defined in chapter 5.11 of the European pharmacopoeia [131].

Table 4–5: Hygroscopicity thresholds defined in [131]

	<i>Upper threshold</i>	<i>Lower Threshold</i>
<i>Deliquescent</i>	N/A	Enough adsorption to form a liquid
<i>Very hygroscopic</i>	N/A	≥ 15 %
<i>Hygroscopic</i>	< 15 %	≥ 2 %
<i>Slightly hygroscopic</i>	< 2 %	≥ 0.2 %

#### 4.2.5 SCANNING ELECTRON MICROSCOPY

Scanning electron microscopy (SEM) is based on the scattering of accelerated electrons. A highly accelerated electron beam (e.g. 10 kV or 15 kV) will yield images with a higher resolution at the expense of a faster sample degradation. Lower voltages (e.g. 5 kV) offer better conditions for surface topography investigations, due to a slower degradation and lower penetration from the electrons into the surface. The electrons interact with the sample, resulting in the emission of backscatter electrons (high energy) and secondary electrons (low energy). Both can be detected with an appropriate detector. The secondary electrons are especially interesting for topographic measurements. SEM analyses are carried out in high vacuums, as this minimises scattering at gas molecules.

Samples were adhered to aluminium stubs with carbon stickers (Plano GmbH, Wetzlar, Germany) and gold coated with a Bal-Tec SCP 005 Sputter Coater (Bal-Tec AG, Balzers, Liechtenstein). SEM images were taken with a PhenomXL (Phenom-World BV, Eindhoven, Netherlands) desktop microscope, equipped with a backscatter detector.

Gold coating the samples was necessary to enhance the conductivity of the sample, as this reduces charging processes in the sample. The sputtering procedure was carried out at 60 mA for 40 seconds, which should theoretically result in a gold layer of about 26 nm [132].

Analyses were carried out at 5 – 15 kV, depending on the respective sample's stability, and in 100 – 25000-fold magnifications.

#### 4.2.6 GAS PYCNOMETRY

Gas pycnometry is a method to evaluate the particle density. The technique, unlike bulk density, takes the open pores of the particles into consideration. Closed pores, however, cannot be evaluated [133].

For this work a helium pycnometer Pycnomatic ATC (Porotec GmbH, Hofheim/Taunus, Germany) was used. In principle, the volume of the sample is equivalent to the volume of the superseded gas, which is temperature dependent. Helium 5.0, which is characterised by a high permeation capability for small pores, was used as the measuring gas.

Samples were weighed into the sample vessel before being placed into the apparatus. The method required the pycnometer to measure each sample for a maximum of 20 cycles. The analysis is stopped before reaching 20 cycles if the standard deviation (SD) of at least five measurements is  $< 0.5\%$ .

### 4.2.7 AERODYNAMIC CHARACTERISATION

The key properties needed to evaluate the performance of a DPI formulation are the delivered dose (DD) and the fine particle dose (FPD), which can be used to calculate the FPF. All these properties are strongly dependent on the used inhaler.

The DD can be evaluated with a dosage uniformity sampling apparatus (DUSA) and the evaluation of the FPD requires impaction analysis, which is considered aerodynamic characterisation.

The European pharmacopoeia names 3 apparatuses (shown in Figure 4–9) to evaluate the fine particle dose in chapter 2.9.18 [134]:

1. Apparatus C, Multi-Stage Liquid Impinger (MSLI)
2. Apparatus D, Andersen Cascade Impactor (ACI)
3. Apparatus E, so called Next Generation Impactor (NGI)

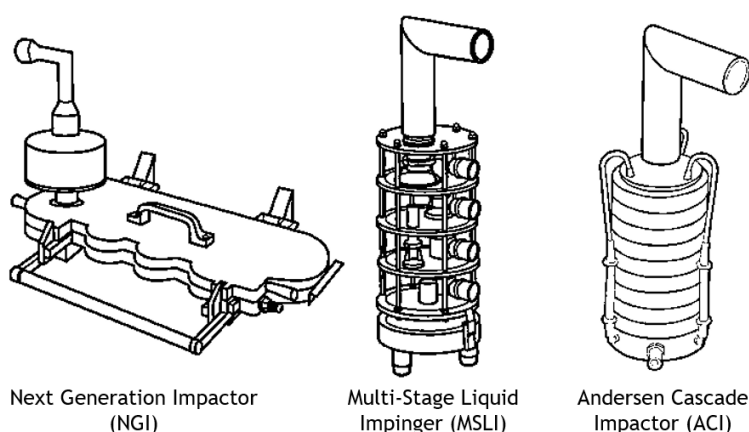


Figure 4–9: apparatuses defined by Ph. Eur. 9.0, NGI (Apparatus E), MSLI (Apparatus C), ACI (Apparatus D) [135]

During this work, the NGI was used for aerodynamic characterisation. It was chosen as it was specifically designed for inhaler testing by the pharmaceutical industry. It is calibrated for

flow rates of 30 – 100 L/min and can also be calibrated for 15 L/min (for nebuliser use). Additionally it is electrically conductive and therefore unaffected by static effects. All of these features are met while the apparatus is simultaneously designed for maximum throughput and easy usability [135].

#### 4.2.7.1 Dosage Uniformity Sampling Apparatus

The DD can be evaluated by delivering one dose (which can require multiple inhalation manoeuvres as a single delivery may not contain the minimum dose) of the formulation with the chosen inhaler into a tube with a terminally placed filter. The API found in the tube, the filter and on the mouthpiece - everything emitted from the device - represents the DD.

For this analysis, the European Pharmacopoeia (*“Zubereitungen zur Inhalation – Inhalanda”*) requires a pressure drop of 4 kPa and an inhaled volume of 4 L [24]. The required parameters for the inhalers were evaluated according to the method described in the Ph. Eur [24]. The required apparatus was built according to the description found in the same chapter.

Table 4–6: Device specific parameters for dose uniformity and impaction analysis

	<i>Cyclohaler</i> <sup>®</sup>	<i>Diskus</i> <sup>®</sup>
<i>Flow rate corresponding to a pressure drop of 4 kPa [L/min]</i>	100	78.1
<i>Measuring time corresponding with a volume of 4 L [s]</i>	2.4	3.1

For the analysis of the formulation with the Cyclohaler<sup>®</sup> one capsule was inserted into the device and pierced before the inhalation manoeuvre was performed with the parameters listed Table 4–6. For the Diskus<sup>®</sup>, two separate inhalations, with the corresponding parameters, were simulated. The reason for this is the filling weight of the blister cup, which is about half the value of the capsules. The capsules were filled to contain one dose. This corresponds to one dose being divided into two blister cups. DUSA analyses were carried out in a controlled environment (21 °C and 45 % rH)

After the manoeuvre, the components of the operation were rinsed with the amounts of solvent listed in Table 4–7. The used solvent was a 3:1 mixture of methanol (MeOH) and aq. bidest. Rinsing took place in a beaker, closed with Parafilm<sup>®</sup> M (Bemis Company, Inc., Neenah, Wisconsin), for one minute on an orbital shaker. The generated samples were analysed with HPLC (see 4.2.8). Due to being a multi-dose inhaler, the Diskus<sup>®</sup> device cannot be rinsed without losing its reusability. Each formulation was analysed before and after the Twelve-Week stability and in triplicate, which is divergent from the Ph. Eur. [24]. Single-dose inhalers, like the Cyclohaler<sup>®</sup> are normally required to be tested ten times, whereas for a multi-dose device like the Diskus<sup>®</sup> the first and last three doses as well as four doses in the middle have

to be tested. This was not possible due to the long run time in the HPLC, as it would have given overlaps with the scheduled sample quantification points. The mean value coupled with the standard deviation of the three analyses is used to describe the dose uniformity of the respective formulation.

Table 4–7: Amount of MeOH:aq. bidest. (3:1) used to rinse the components of the inhalation manoeuvre (DUSA)

<i>Component</i>	<i>Cyclohaler®</i>	<i>Diskus®</i>
<i>Device</i>	10 mL	N/A
<i>Capsule</i>	10 mL	N/A
<i>Mouthpiece + Filter</i>	10 mL	10 mL
<i>Tube</i>	15 mL	15 mL

The API recovery has a large influence on the results of this analysis. Therefore, the results were made comparable by relating the DD to the recovery and thus calculating the adjusted delivered dose of each run.

$$\text{Adjusted Delivered Dose} = \frac{\text{Delivered Dose } [\mu\text{g}]}{\text{Recovery } [\mu\text{g}]} \cdot 100 \% \quad (4-2)$$

### 4.2.7.2 Next Generation Impactor

As mentioned in 4.2.7, the NGI is an apparatus used for impaction analysis. Impaction analysis is based on inertial deposition of the particles in a curved airstream (see chapter 2.2). Due to increasing airflow velocities and a higher curvature of the airstream with each successive stage, progressively smaller particles will deposit [28]. The increase of the airflow velocity is achieved by reducing orifice diameters, while the higher curvature is gained by moving the nozzles closer to the respective stage. The applied flow rate dictates the cut-off value (size) of the stages. Five stages have cut-off diameters between 0.5 and 6.5  $\mu\text{m}$  for every flow rate (30 – 100 L/min). The cut-off diameter is defined as the diameter that has a deposition probability of 50 % [134].



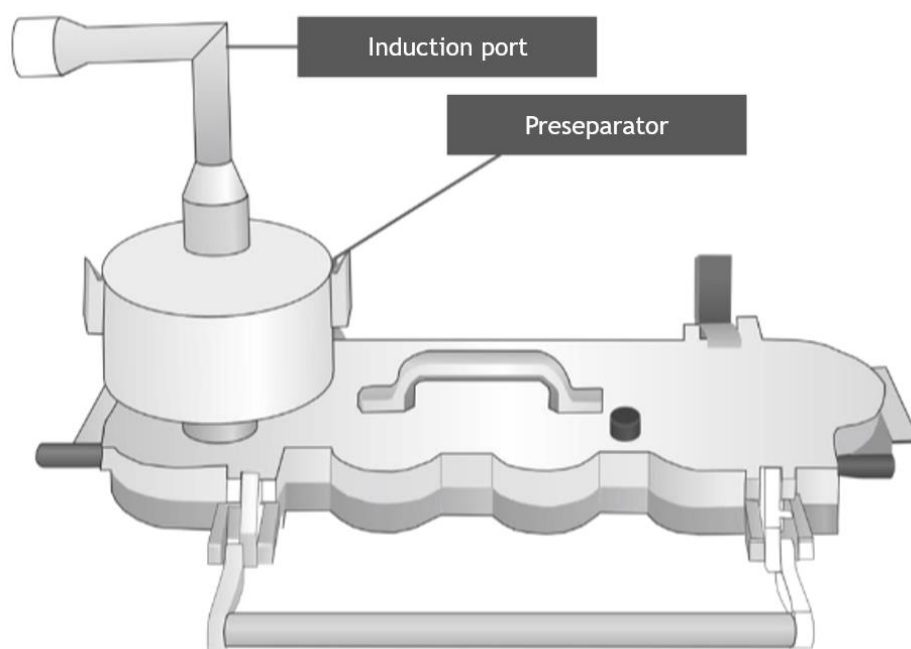


Figure 4–10: NGI (closed) schematic, with preseparator (connected to stage 1) and induction port (“throat”)

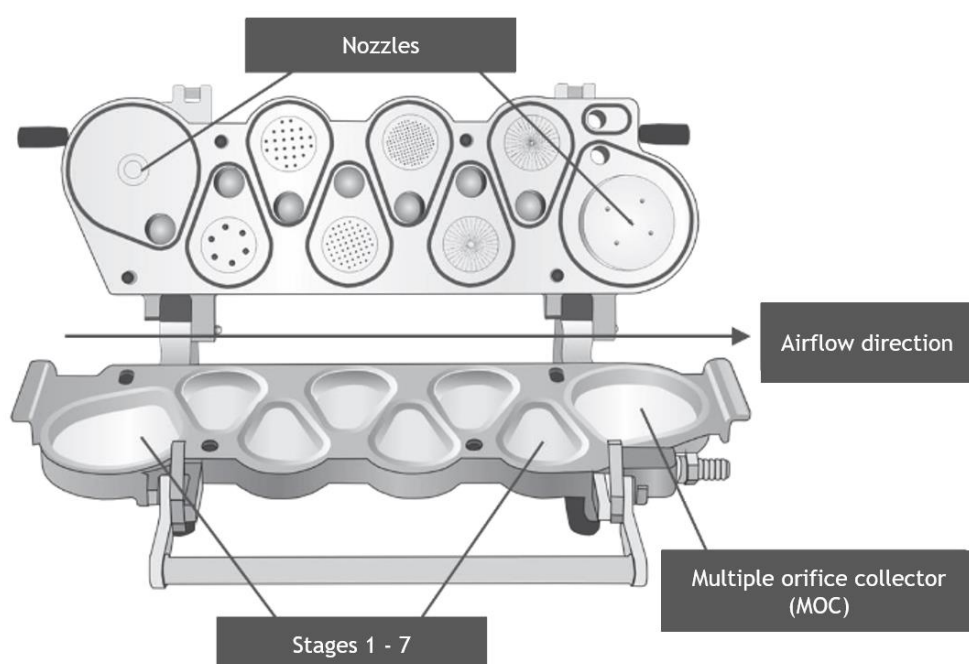


Figure 4–11: NGI (open) schematic, seven stages and Multiple Orifice Collector (MOC) (bottom), corresponding nozzles in the cover (top)

The NGI (Figure 4–10 & 11; Copley Scientific Limited, Nottingham, UK) is a multistage impactor which can be augmented by a preseparator (Figure 4–10), which is especially useful to separate larger carrier particles from interactive blends. The knowledge of the applied airflow enables the calculation of the particle size classes which will impact on the individual stages.

The NGI is constructed in a way that in a flow-rate range of 30 – 100 L/min at least five stages have a deposition diameter between 0.5 – 6.5  $\mu\text{m}$  [134].

For this work the preseparator was used, although no carrier particles were added to the formulation, to prevent larger agglomerates, which would normally impact in the oropharynx, from impacting on stage 1.

The analysis was carried out as described in chapter 2.9.18 of the Ph. Eur. [134], with the exception of adding the solvent (see 4.2.7.1) to the preseparator before the analysis. Instead, the preseparator was coated, like the stages and the MOC, with a mixture of Brij<sup>®</sup> 35 (polyoxyethylene (23) lauryl ether), EtOH and glycerol (51:15:34). This coating is used to prevent the particles from bouncing from the stages' surfaces and being reclaimed by the airstream. Like DUSA analyses, analyses were carried out in a controlled environment.

Measurements were carried out with the parameters described in Table 4–6. After the inhalation manoeuvre (two capsules for the Cyclohaler<sup>®</sup>, four shots for the Diskus<sup>®</sup>) the components were rinsed with the amount of solvent (MeOH:aq. bidest. 3:1) shown in Table 4–8. The stages and MOC were covered with an acrylic glass plate while the capsule, device and mouthpiece were put into beakers and covered with Parafilm<sup>®</sup> M. The throat was covered with an endcap and Parafilm<sup>®</sup> M and the preseparator with rubber plugs. The preseparator was shaken by hand while the stages and other components were shaken on an orbital shaker for one minute. These samples were analysed with HPLC (see 4.2.8). Each formulation was analysed in triplicate.

Table 4–8: Amount of solvent used to rinse the components of the inhalation manoeuvre (NGI)

<i>Component</i>	<i>Cyclohaler<sup>®</sup></i>	<i>Diskus<sup>®</sup></i>
<i>Capsule</i>	10 mL	N/A
<i>Device</i>	10 mL	N/A
<i>Mouthpiece</i>	10 mL	10 mL
<i>Throat</i>	15 mL	15 mL
<i>Preseparator</i>	20 mL	20 mL
<i>Stage 1 - 7</i>	5 mL	5 mL
<i>MOC</i>	5 mL	5 mL

Data evaluation was carried out with the CITDAS 3.0 software (Copley Scientific Limited, Nottingham, UK). The software calculates the cut-off diameter of each stage in dependency of the applied flowrate. Plotting the cumulative amount of API against the common logarithm of

the cut-off diameter of the corresponding stage enables the determination of the mass median aerodynamic diameter (MMAD). The FPD (mg or  $\mu\text{g}$ ) is defined as the cumulative mass of every particle in the inhalable range (i.e.  $<5\ \mu\text{m}$  [14]).

$$\text{Fine Particle Fraction} = \frac{\text{Fine Particle Dose}}{\text{Delivered Dose}} \quad (4-3)$$

Equation (4–3) shows how the FPF can be calculated from the FPD. The FPF is often used as a characteristic to describe the dispersion efficiency of inhalers, for example by de Boer et al. in 2016 [42], although it is also dependent on the formulation. The FPF does not consider the amount of drug retained in the device. Both the FPF and FPD were used to evaluate the formulations and suitability of the used inhalers.

The Ph. Eur. specifies a recovery of 75 – 125 %, compared to the recovery of dose uniformity measurements, for NGI measurements [134].

#### 4.2.7.3 Fast Screening Impactor

The Fast Screening Impactor (FSI) is an apparatus designed by Copley Scientific (Nottingham, UK) to enable a faster evaluation of the fine particle dose than cascade impactor analyses. Due to the setup of the FSI, an evaluation of the MMAD is not possible. The FSI combines the typical preseparator from the NGI with a different perforated plate. The used plate is designed in a way that particles with an aerodynamic size of  $> 5\ \mu\text{m}$  will deposit in the preseparator while the fine particle dose passes the preseparator and deposits in a terminally placed filter. A disadvantage of the FSI, however, is the requirement of a specific perforated plate for each flowrate.

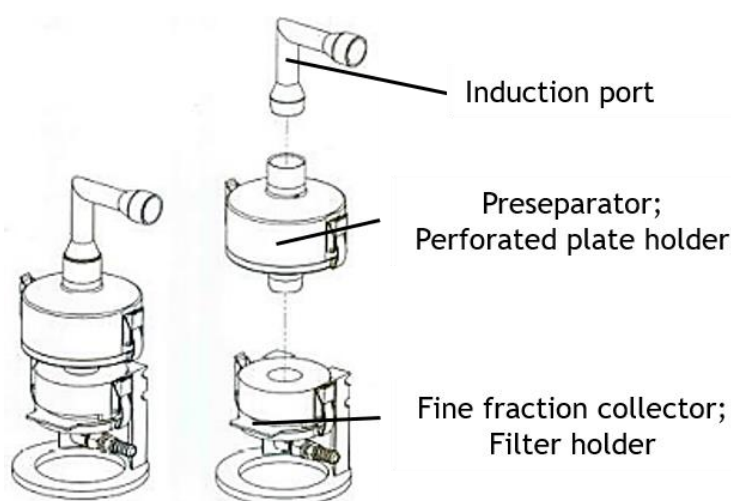


Figure 4–12: Schematic diagram of the FSI

According to the Ph. Eur., pMDIs require a flowrate of 30 L/min [134]. As no perforated plates for the required flowrates of the DPIs were available, the FSI was only used for the analyses of the FPFs and FPDs of the pMDIs in chapter 5.1.

Analyses were carried out in a controlled environment (21 °C and 45 % rH) and in triplicate. Each sample was actuated twice into the FSI. The samples were rinsed with the amount of solvent defined in Table 4–9, rinsing was carried out analogous to the corresponding sample type for the NGI analyses.

Table 4–9: Amount of solvent used to rinse the components of the inhalation manoeuvre (FSI)

<i>Component</i>	<i>MeOH:aq. bidest. (3:1)</i>
<i>Device</i>	10 mL
<i>Mouthpiece</i>	10 mL
<i>Throat</i>	15 mL
<i>Presparator</i>	20 mL
<i>Filter</i>	10 mL

### 4.2.8 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

High performance liquid chromatography (HPLC) is a form of column chromatography. Here, the mobile phase is pressed through the column by pumps. HPLC is generally used to separate substance mixtures and for the qualitative and quantitative analysis of the individual components. Results are displayed as chromatograms, which is a diagram where the intensity of the detector signal is plotted against the time. HPLC offers distinctly higher separation efficiency than conventional column chromatography. Due to almost arbitrary combination possibilities of columns (e.g. Column material, separation mechanism) and mobile phases, HPLC can separate virtually every soluble organic or inorganic drug [128].

For this work an Agilent Series 1100 HPLC (Agilent Technologies Inc., Santa Clara, USA), equipped with a reversed phase column was used. The utilised methods are defined in chapter 10.4. A gradient method was developed during the MS analytics for chapter 5.2.1.1. Therefore, all the results starting from this chapter were acquired with the gradient method.

The experiments to identify the main degradants (chapter 5.2.1.1) were carried out with an Agilent Series 1200 HPLC (Agilent Technologies Inc., Santa Clara, USA) coupled with a Bruker Amazon SL Ion Trap mass spectrometer (MS; Bruker Daltonics, Bremen, Germany). Substances eluted between two and twelve minutes were fed to the MS. Due to its sensitivity, the injection volume was reduced to 0.1 µL for these analyses.

Quantification has been performed by an external standard calibration utilising the CBD batch (Canapure<sup>®</sup>, 18R18786) with its declared drug content of 100.5 % [136]. For the analyses the content was assumed to be 100 %.

### 4.3 STABILITY TESTING

#### 4.3.1 SHORT TERM STRESS TESTS

##### 4.3.1.1 UV-Irradiation

Individual ethanolic solutions of AO (AA, AP, BHA, BHT) and CBD in different molar ratios (0.5:1, 1:1, 2:1 and 10:1) were prepared so that the CBD concentration was 80 µg/mL. Each solution as well as a pure CBD solution was filled in a disposable UV-cuvette (Sarstedt AG & Co. KG, Nürnbergrecht, Germany), which was subsequently closed with a cap. Each cuvette was placed in the UV chamber at an angle of about 45 ° to ensure efficient radiation (Figure 4–13). The samples were irradiated for 24 hours at 254 nm. The CBD content was analysed with HPLC after 3 and 24 hours. In addition to the resulting 16 samples, two reference samples without an AO were analysed in parallel. One reference was treated like the samples; the other was stored in the refrigerator.

This analysis was carried out at room temperature and in triplicate.

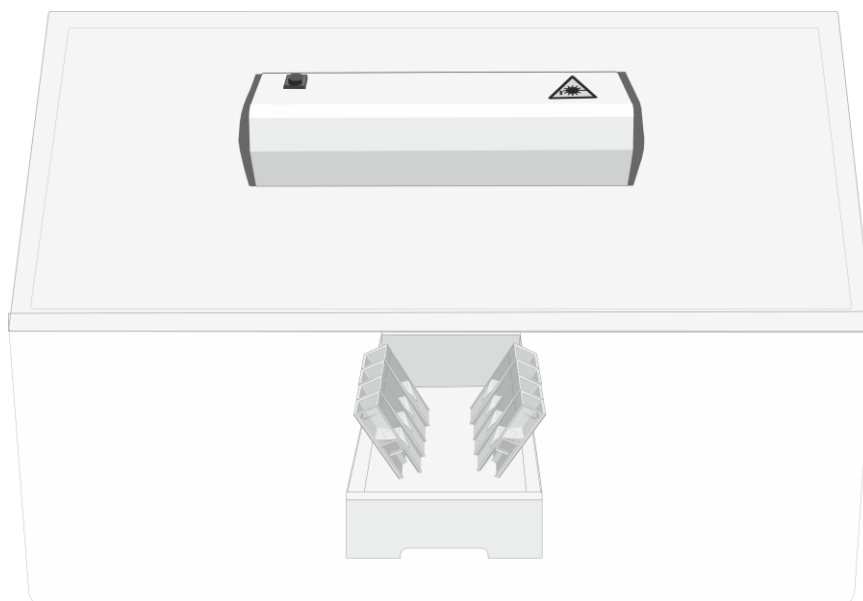


Figure 4–13: Buildup of the UV irradiation chamber

##### 4.3.1.2 Temperature Stress

Ethanolic solutions of CBD and resinous CBD with a concentration of 75 µg/mL were prepared and placed in tightly closable glass bottles. These bottles were stored at 60 °C, 80 °C and 100 °C for 24 hours and in darkness. Taking the samples required opening of the bottle, which naturally lead to the loss of the gaseous EtOH. The escaped/evaporated amount of EtOH was measured with a graduated cylinder and replaced. Samples were analysed for their CBD content after 1, 3 and 24 hours by HPLC. This analysis was done in triplicate.

### 4.3.2 TWELVE-WEEK STABILITY

#### 4.3.2.1 Storage Conditions

Storage conditions for the Twelve-Week stability study were adapted from ICH guideline Q1A(R2) [137] and are shown in Table 4–10. The samples were placed in the respective storage chamber on three successive days to take the duration of the content analyses into account.

Table 4–10: Storage conditions for the Twelve-Week stability study

<i>Storage Condition</i>	<i>Temperature [°C]</i>	<i>Relative Humidity [%]</i>	<i>Storage Apparatus</i>
<i>1 (fridge)</i>	$5 \pm 3$	N/A	Refrigerator
<i>2 (25/60)</i>	$25 \pm 2$	$60 \pm 5$	Climate cabinet
<i>3 (40/75)</i>	$40 \pm 2$	$75 \pm 5$	Climate cabinet

The storage conditions were monitored and registered externally with a KLIMALOGG PRO Professional Thermo-Hygrometer with Data Logger Function (TFA Dostmann GmbH & Co. KG, Wertheim-Reicholzheim, Germany) and three external temperature-humidity transmitters. Both the rH and the temperature were logged every 30 minutes.

#### 4.3.2.2 Samples

The samples stored at each storage condition are listed in Table 4–11. To enable subsequent analytics, a sufficient number of samples was stored. The solutions were prepared with HPLC grade EtOH (absolute, 99.9 wt%) as the solvent.

Table 4–11: Samples for Twelve-Week stability study

<i>Sample</i>	<i>Storage Vessel</i>	<i>Total samples</i>
<i>Spray-dried formulations (6 formulations)</i>	Open Eppendorf tube, closed capsule, blister strip	18
<i>Crystalline CBD</i>	Open Eppendorf tube	1
<i>Resinous CBD</i>	Open Eppendorf tube	1
<i>CBD pMDI</i>	pMDI can	1
<i>CBD pMDI with AO</i>	pMDI can	1
<i>CBD solution</i>	Snap cap glass vessel (SCG)	1
<i>CBD solution with AO</i>	SCG	1

4.3.2.3 *Sample Preparation for Content Analysis*

Samples were evaluated for their CBD content directly after preparation and after 1, 2, 3, 4, 8 and 12 weeks. After 12 weeks the samples, with the exception of the pMDIs, were irradiated with 254 nm UV light for five days and again analysed quantitatively. The samples were prepared as stated in Table 4–12 with acetonitrile:aq. bidest. (85:15) as the solvent.

Table 4–12: Preparation of individual samples of Twelve-Week stability for content analysis

<i>Sample</i>	<i>Analysis Sample Preparation Procedure</i>
<i>Spray-dried formulations, Eppendorf tube</i>	Tube put into SCG; 20 mL solvent added; 30 mins ultrasonication
<i>Spray-dried formulations, Capsule</i>	Capsule opened; put into SCG; 20 mL solvent added; 30 mins ultrasonication
<i>Spray-dried formulations, Blister strip</i>	Blister strip opened; powder weighed into SCG; 5 mL solvent added; thorough shaking
<i>Crystalline CBD</i>	Tube put into SCG; 20 mL solvent added; thorough shaking; 1:10 dilution with solvent
<i>Resinous CBD</i>	Tube put into SCG; 20 mL solvent added; thorough shaking; 1:10 dilution with solvent
<i>pMDIs</i>	pMDI primed; 1 shot actuated into HPLC vial; 1 mL solvent added; 1:20 dilution with solvent
<i>Solutions</i>	1:20 dilution with solvent

Content analyses were done in triplicate.



## 4.4 STATISTICAL METHODS

### 4.4.1 DESIGN OF EXPERIMENTS

An experimental design (Design of Experiments, DoE) is an experimental set-up to simultaneously evaluate several factors at a given number of levels in a predefined number of experiments. DoEs can be divided into screening designs (e.g. full factorial), response surface designs and mixture designs. Screening designs offer the possibility of identifying the most influential factors by screening a large number of factors in relatively few experiments. Response surface designs can be used to evaluate the optimal levels of the important factors, they are therefore often used subsequently to a screening. A mixture design is a more complex version of the response surface design, which has to be used when the factors are mixture-related (e.g. excipients in a pharmaceutical formulation) [138].

DoEs done in this work were established and evaluated with the software Modde (version 10.1, Umetrics, Malmö, Sweden). The individual designs done for this work are described more closely in chapters 5.1.2 ( $2^3$  full factorial), 5.3.1 ( $3^3$  full factorial) and 5.4.1 (L18 fractional factorial design).

### 4.4.2 GENERAL STATISTICAL ANALYSIS

Mean values and standard deviations were calculated with equations (4–4) and (4–5). The requirement for this is the assumption that the evaluated data sets are normally distributed. Normal distribution was checked exemplary with SigmaPlot (version 11.2.0.5, Systat Software GmbH, Erkrath, Germany). For graphical presentation of data, the relative mean value (related to the initial value) will be used while the relative standard deviation (RSD) will be depicted as error bars. In the running text the standard deviation will be indicated by the  $\pm$ -symbol. Calculation of the RSD was achieved with equation (4–6).

$$\bar{x} = \frac{\sum_{i=1}^n x_i}{n} \quad (4-4)$$

$\bar{x}$	Arithmetic mean
$n$	Sample size
$x_i$	i-th value of the variable $x$

$$SD = \sqrt{\frac{\rho \sum_{i=1}^n (x_i - \bar{x})^2}{n - 1}} \quad (4-5)$$

$SD$	Standard deviation
------	--------------------

$$RSD = \frac{SD}{\bar{x}} \cdot 100 \% \quad (4-6)$$

*RSD*            Relative standard deviation

Analysis of statistical values was carried out with Microsoft Excel (version 2016 & 365, Microsoft Corporation, Redmond, WA, USA). Significant differences between results were identified implementing an f-test followed by an unpaired two-sided *t*-test. If the variances were similar (result from the f-test) students' *t*-test was used, while a *t*-test for dissimilar variances was applied if the variances were significantly different.

For this work *p*-values of  $0.05 > p \geq 0.01$  are considered significant (\*) while values of  $0.01 > p \geq 0.001$  are considered highly significant (\*\*) and values  $0.001 > p$  are considered most significant (\*\*\*).

## 5 RESULTS AND DISCUSSION

### 5.1 *CBD PMDIs*

The evaluation of the performance of marketed recreational pMDIs was identified as an important aspect to determine the potential of this formulation type in a medical setting.

#### 5.1.1 *COMPOSITION*

As mentioned in chapter 1.1, Mystabis and the Quest Aero™ inhaler™ are two marketed recreational cannabinoid inhalers. Both producers claim that their devices are of pharmaceutical grade and Mystabis advocates only using the inhaler if it has been recommended by a physician. According to the respective websites, the formulations contain HFA 134a, EtOH and natural cannabis extracts (Mystabis [139]) or cannabis oil (Aero™ inhaler [140]). Quest also states that one puff (100 µl) of the Aero™ inhaler administers 5.0 mg of “activated cannabinoids”. This corresponds to a cannabinoid concentration of 50 µg/µL.

As the marketed products are only available in the US and import was impossible due to legal restrictions, own formulations have been developed, based on known characteristics of the commercial products.

In his doctoral thesis, Sven Stolte showed that higher amounts of EtOH in a pMDI formulation lead to reduced FPDs. This is caused by a change in the vapour pressure, which in turn changes the exit velocity and the size of the droplets [141].

Table 5–1: Quantitative composition of pMDIs for aerodynamic performance

<i>Formulation</i>	<i>CBD [mg]</i>	<i>Ethanol [mL]</i>	<i>HFA 134a [g]</i>
<i>10 mg/mL</i>	120	1	13.31
<i>30 mg/mL</i>	360	1	13.31
<i>50 mg/mL</i>	540	1	13.31

Based on the concentration and components of the Aero™ inhaler formulation and Stolte’s findings, a formulation with 50 µg/µL CBD with the least possible amount of EtOH to dissolve the drug was developed. Two additional formulations with lower CBD concentrations were additionally produced in order to evaluate if a lower concentration and therefore smaller particles after propellant evaporation leads to a better performance. The composition of the formulations is shown in Table 5–1. Each formulation had an EtOH content (in the solvent/propellant mixture) of 8.3 %.

### 5.1.2 EXPERIMENTAL DESIGN TO EVALUATE THE AERODYNAMIC PERFORMANCE

A DoE to maximise the aerodynamic performance of the different formulated pMDIs was implemented. The variable parameters (Table 5–2) for this DoE were the theoretical dose (61 µL valve) and the orifice diameter. Every possible combination (2<sup>3</sup> full factorial) of these parameters was tested in triplicate, to assess the reproducibility. This resulted in 27 runs (Table 10–2). The critical outcome of this DoE was the FPF and the goal was the maximisation of the efficiency of the pMDI formulation. The formulation with the ability to apply the desired dose (0.83 – 3.33 mg, see chapter 3.1.1) with the fewest actuations while maintaining a high efficiency (FPF), to minimise potential costs, was considered optimal (includes the orifice).

Table 5–2: Variable parameters and levels for the pMDI DoE

<i>Parameter</i>	<i>Level 1</i>	<i>Level 2</i>	<i>Level 3</i>
<i>Theoretical Dose [mg]</i>	0.61	1.83	3.05
<i>Orifice Diameter [mm]</i>	0.22	0.30	0.42

Table 5–3 shows the achieved FPF of each parameter combination. The significance of the differences between the optimum (max. FPF; 0.61 mg & 0.22 mm) and every other combination were evaluated and marked, as described in chapter 4.4.2. It is visible that a smaller orifice diameter as well as a lower theoretical CBD dose increases the FPF. This can also be seen in Figure 5–1.

Table 5–3: Mean FPF and standard deviation of CBD pMDIs; n = 3; n = 2, outlier excluded

	<i>0.22 mm orifice</i>	<i>0.30 mm orifice</i>	<i>0.42 mm orifice</i>
<i>0.61 mg</i>	35.1 ± 2.5 %	24.7 ± 3.4 %*	22.3 ± 2.5 %*
<i>1.83 mg</i>	34.0 ± 2.4 %	21.1 ± 2.2 %***	20.4 ± 2.0 %***
<i>3.05 mg</i>	28.6 ± 2.9 %*	19.7 ± 2.5 %***	20.0 ± 1.7 %***

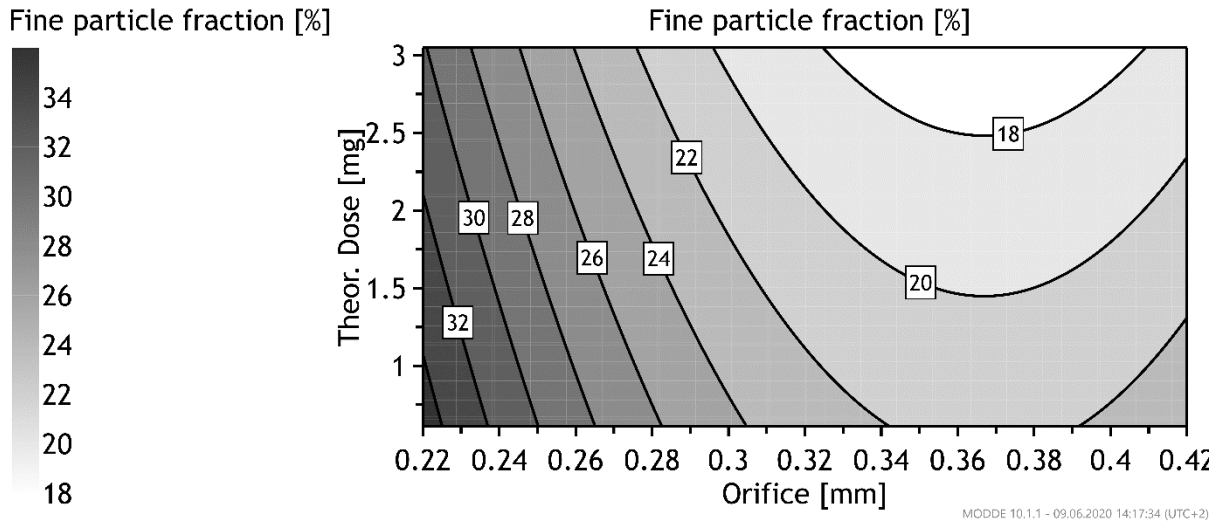


Figure 5–1: Response contour plot of the FPF of CBD pMDIs

Modde summarises the fit of a model with four different parameters (max. value = 1.0): R2, Q2, model validity, reproducibility.

R2 shows the model fit and should be  $> 0.5$ ; a value  $< 0.5$  signals a rather low significance. The generated model had a R2 of 0.86.

Q2 is an estimate of the future prediction precision. It should be  $> 0.1$  for a significant model and  $> 0.5$  for a good model. The software claims that Q2 is the best and most sensitive indicator. The generated model had a Q2 of 0.81.

The model validity is a test of diverse model problems. If the value for this parameter is  $< 0.25$  it indicates statistically significant model problems, for example outliers, an incorrect model or a transformation problem. It could also indicate that a term, like an interaction or square of the input parameters is missing. However, if the pure error is very small, the model validity can be low although the model is good. The model validity of this DoE was 0.87.

The reproducibility is the variation of the replicates compared to the overall variability. The value for this parameter should be  $> 0.5$ . The generated model had a reproducibility of 0.84.

Every parameter of the summary of fit was distinctly higher than the values which are considered as the lower threshold for a good model, which shows that the generated model was very good.

Interestingly, the model predicts a resurgence of the FPF after reaching a minimum for orifices with a diameter of about 0.36 mm, especially for higher theoretical doses. This is probably due to the minimum of the datapoints (3.05 mg & 0.30 mm). However, as the difference of the FPF between this combination and the 3.05 mg & 0.42 mm is insignificant ( $p = 0.623$ ), this could be a mistake in the generated model.

The medium dose coupled with the smallest orifice can be labelled as the most efficient of the nine formulations in delivering CBD to the lungs, as the difference to the low dose coupled with the smallest orifice is insignificant. Therefore, the medium dose with the smallest orifice was chosen for the stability study.

### *5.1.3 STABILITY*

Glass cans were used during formulation development of the pMDIs, instead of aluminium cans. This made it possible to see if the drug was completely dissolved during production or if precipitation occurred after adding the propellant or during storage time.

No precipitation could be observed after preparation. However, after several weeks of environmental storage (with sun exposure) a discolouration was noticeable. The originally colourless formulation slowly changed over a light yellow with increasing amounts of violet and finally reached an auburn colour. No precipitation was observable over this storage.

These signs of obvious instability were the reason for further stability experiments of CBD in solution and the decision to carry out a prolonged, twelve-week stability trial.

## 5.2 SHORT TERM CBD STABILITY STRESS TESTS

### 5.2.1 INFLUENCE OF UV-IRRADIATION ON ETHANOLIC CBD SOLUTIONS

The following chapter covers the results of two different types of experiments that are based on UV catalysed reactions of dissolved CBD.

#### 5.2.1.1 Experiments to Identify Degradation Products

The pMDI formulation developed an increasing amount of violet this is a colour observed by Mechoulam and Hanuš [8] for hydroxyquinones during the oxidation of CBD (Figure 5—2). Additionally, several light induced (Figure 5—3 & 4) reactions were described in this publication. Therefore, it was decided to investigate if any main degradants could be identified by utilising advanced analytics.

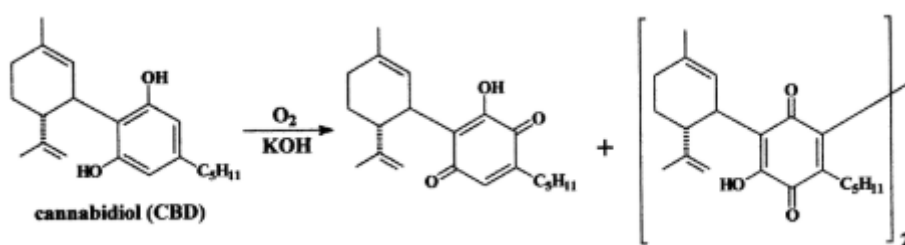


Figure 5—2: Quinone formation from CBD [8]

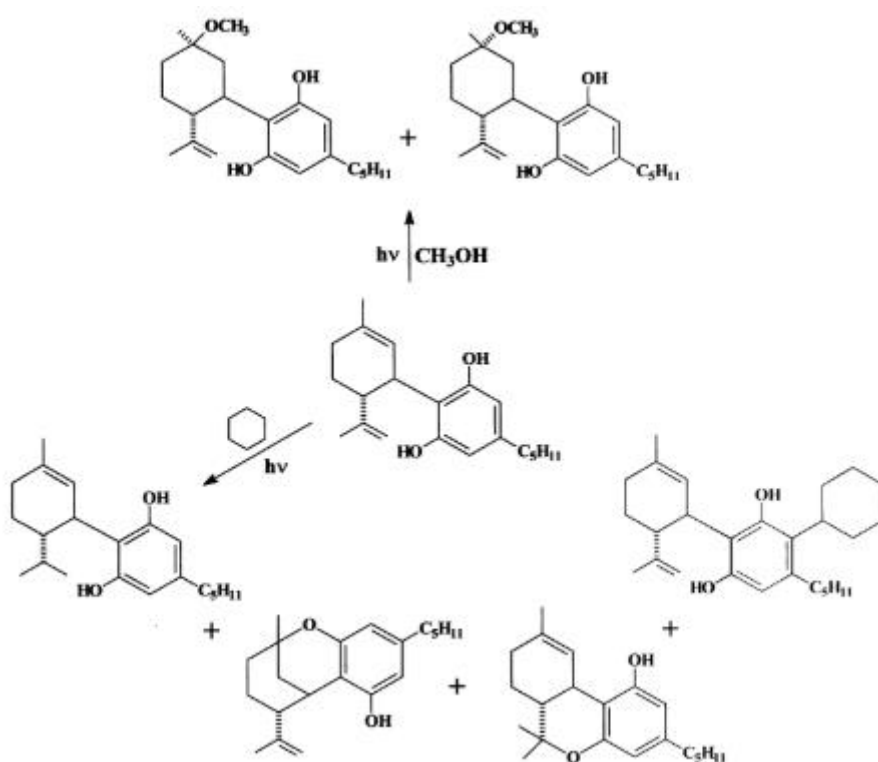


Figure 5—3: Photochemical reactions of CBD [8]

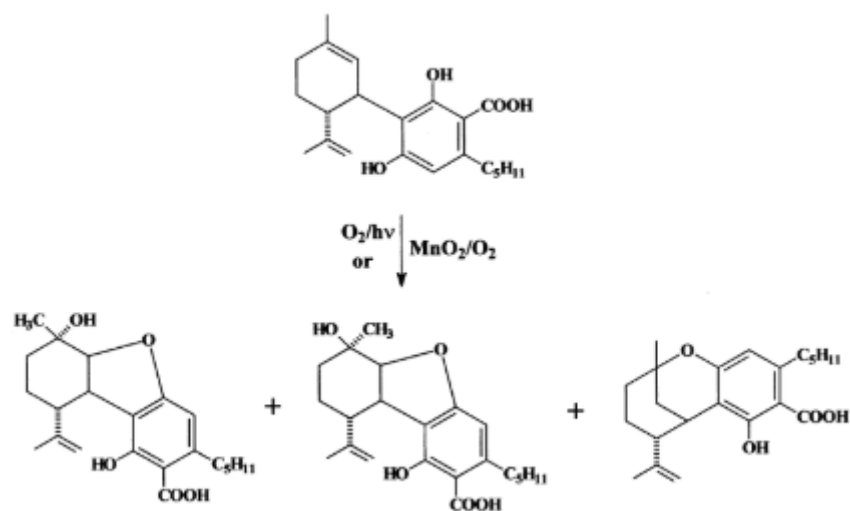


Figure 5–4: Formation of cannabielsoic acid-type compounds from CBD [8]

These trials were carried out with HPLC-MS. To achieve a better separation of CBD and its degradants, the HPLC method was adjusted from an isocratic method to a gradient method (see chapter 10.4).

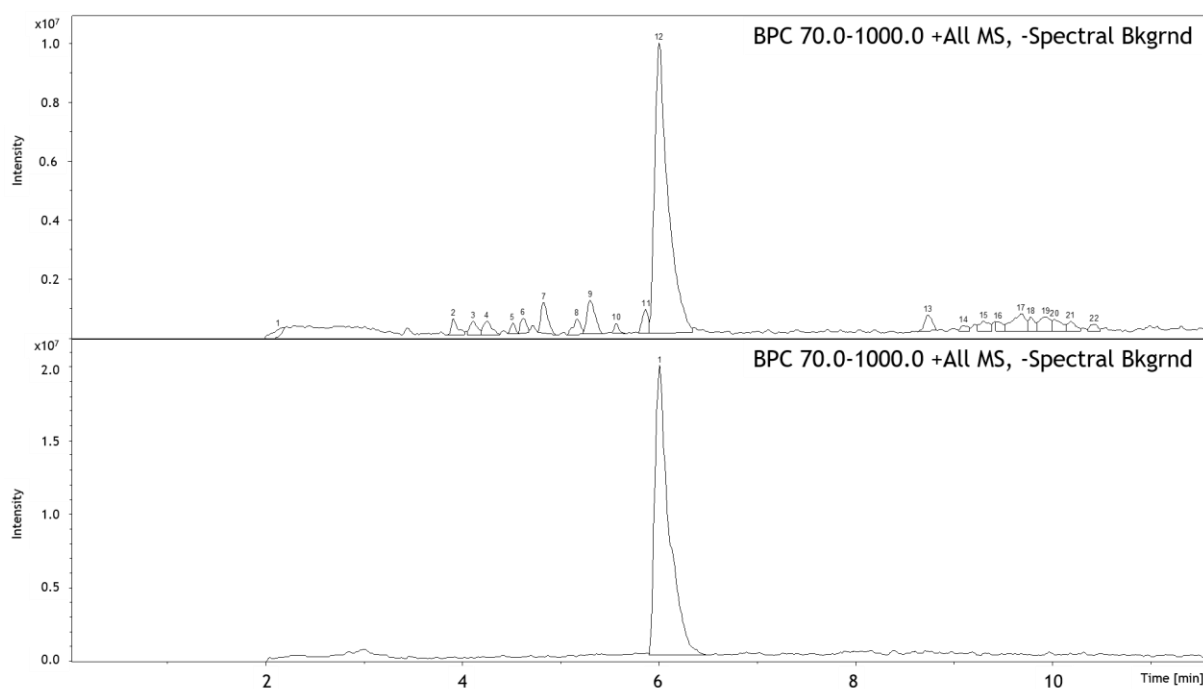


Figure 5–5: Base peak chromatograms (HPLC-MS) of a UV-irradiated CBD solution (top) and a fresh CBD solution (bottom); Intensity = detector signal of the MS

To get high concentrations of degradants, 10 mg/mL CBD solutions (pure CBD in EtOH) were prepared and, as a photo degradation (environmental storage) or an oxidation (violet colour) was seen as the most probable reaction, irradiated with 254 nm for 24 hours in a UV-cuvette at room temperature (analogous to the method described in chapter 4.3.1.1). The samples



developed a yellow colour during the UV irradiation (compare with 5.1.3). This was seen as a confirmation of the photodegradation, which is consistent with Mechoulam and Hanuš [8]. MS results, which were generated in cooperation with Dr. Ulrich Girreser from the institute of pharmaceutical chemistry, however, allowed no identification of main degradants, as a multicomponent mixture (22 auto-detected peaks in the MS, highest Peak = CBD, Figure 5—5) was created through UV irradiation. This matches the observations made in a patent for stable cannabinoid compositions [142]. In this patent it is shown that HPLC-analysis of a CBD solution without stabilisers resulted in a decrease of the CBD peak while several overlapping peaks are gained before the CBD peak, after the solution was stored for 275 days under natural light exposure.

Concluding, these trials showed that an identification of CBDs main degradants is not easily achievable. Therefore, the stability of CBD was evaluated by calculating the reduction of its concentration (i.e. main HPLC peak (UV detection)) in solution. As it was found out to be easier to separate CBD and its degradants, the gradient method was used from this point.

#### 5.2.1.2 *Experiments to Identify a Suitable Antioxidant*

To evaluate if the degradation of CBD in a solid solution can be reduced by adding a suitable AO, the experiment described in chapter 4.3.1.1 was developed. This experiment was carried out in solution, due to the generally reduced stability in solutions.

For this experiment four different AOs were evaluated (AA, AP, BHA and BHT). AA and AP were chosen as both have been successfully used to stabilise cannabinoids (see chapters 3.1.3.1 & 3.1.3.2) and have different lipophilicities. BHA and BHT were chosen as they are commonly used in pharmaceutical preparations and as they are relatively lipophilic (see chapters 3.1.3.3 & 3.1.3.4), which is important as CBD is extremely lipophilic.

The different ratios (0.5:1, 1:1, 1:2, 1:10) were chosen to gain an overview on the needed amount of AO, as this also has an influence on the following formulation of a solid dispersion. An AO that needs to be used in a 10:1 ratio to achieve the desired amount of protection reduces the maximum amount of CBD, which can be used in the developed formulation.

The goal of this trial was the identification of the best suited AO. It was chosen to decide this on the basis of the protecting capabilities as well as the lipophilicity and possibly the safety, if two substances were to be comparable in the other aspects.

Figure 5—6 to Figure 5—10 show the development of the CBD concentration over 24 hours. The 1:1 ratio of AA or AP achieved about the same level of protection as the 10:1 ratio of BHA or BHT, indicating more potent protecting capabilities for AA and AP. The 10:1 ratios of AA and AP achieved close to perfect protection over 24 hours. Interestingly, the 2:1 ratio of BHA and

## Short Term CBD Stability Stress Tests

BHT reached the lowest end concentration. The order of the end concentrations for AA and AP is expected - a higher ratio achieves a higher end concentration.

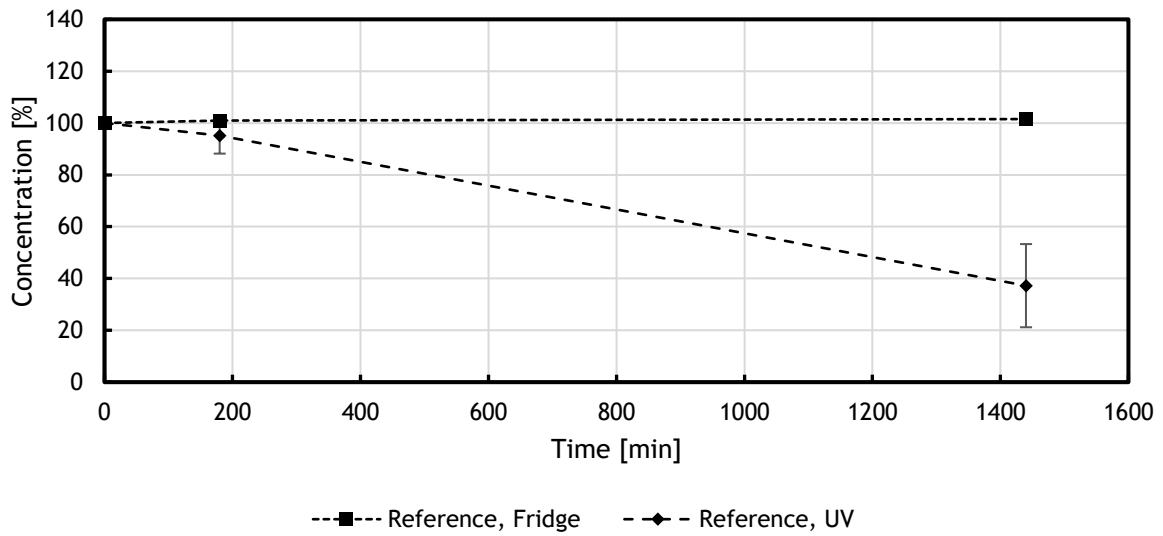


Figure 5-6: Concentration (CBD peak) development of reference (CBD without AA) samples;  $n = 3$ ; error bars = SD

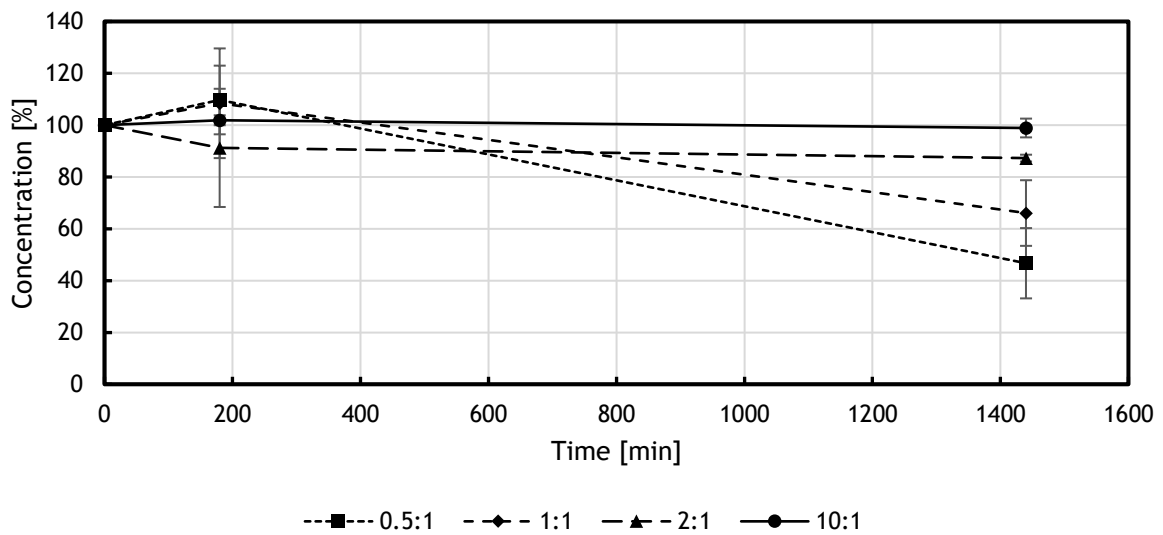


Figure 5-7: Concentration (CBD peak) development of CBD solutions with AA in different ratios;  $n = 3$ ; error bars = SD

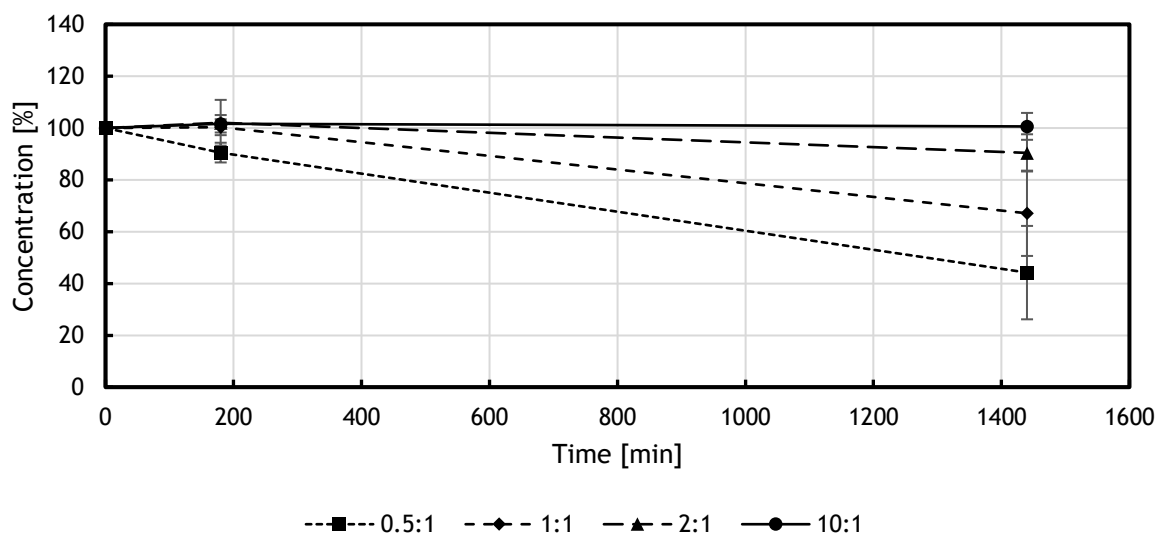


Figure 5-8: Concentration (CBD peak) development of CBD solutions with AP in different ratios;  $n = 3$ ; error bars = SD

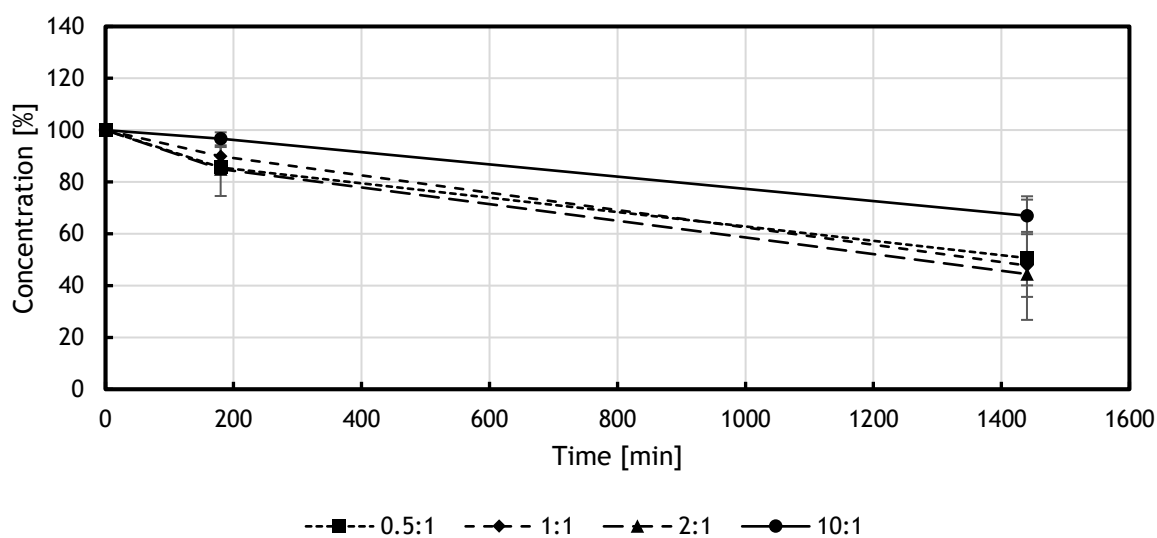


Figure 5-9: Concentration (CBD peak) development of CBD solutions with BHA in different ratios;  $n = 3$ ; error bars = SD

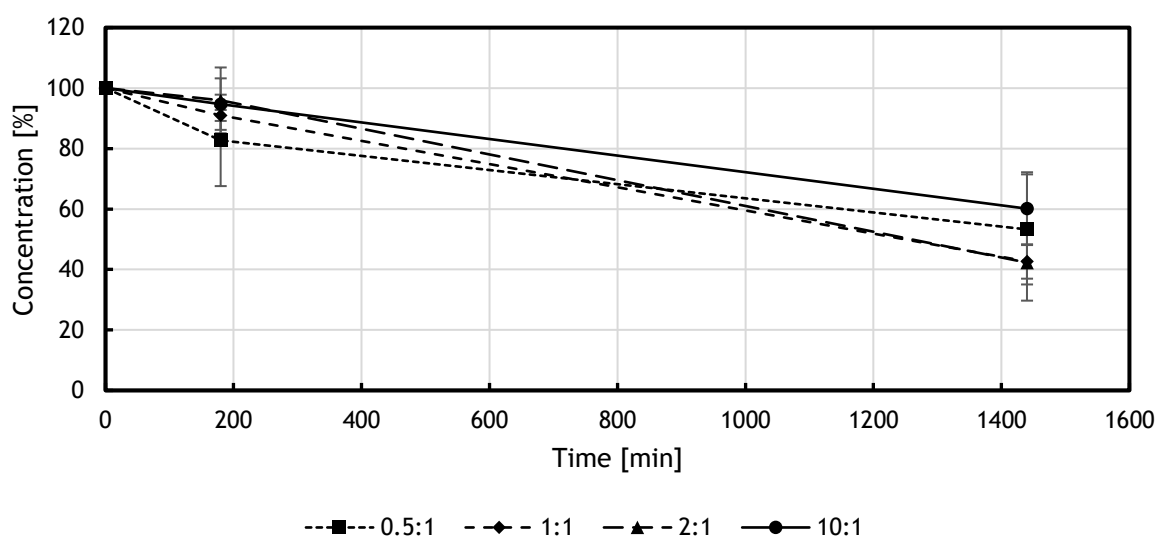


Figure 5-10: Concentration (CBD peak) development of CBD solutions with BHT in different ratios;  $n = 3$ ; error bars = SD

The most relevant statistical analyses showed that the differences between the concentrations of AA (10:1) and AP (1:1, 2:1, 10:1) and the refrigerated reference after 24 h were insignificant ( $p > 0.05$ ), while BHA (exception 0.5:1,  $p = 0.066$ ) and BHT reached significantly ( $p < 0.044$ ) lower concentrations than this reference after 24 h.

Compared to the irradiated reference, AA and AP, in a 2:1 or 10:1 ratio, achieved significantly ( $p < 0.033$ ) higher end concentrations. This was also the case for BHA in a 10:1 ratio ( $p = 0.040$ ).

The 10:1 ratio of AA was better ( $p < 0.012$ ) in protecting CBD than the other three, while the 2:1 ratio was more effective ( $p = 0.036$ ) than the 0.5:1 ratio. With the exception of the difference between the 10:1 and the 2:1, which was insignificant ( $p = 0.116$ ), AP behaves analogously ( $p < 0.028$ ). After 24 h the 2:1 ratio of BHA was worse ( $p = 0.007$ ) than its 10:1 ratio, while the differences between the other ratios were insignificant ( $p > 0.071$ ). For BHT differences between each ratio were insignificant ( $p > 0.086$ ) after 24 h.

As a result of these experiments, AP was chosen as the most promising AO for formulation studies. The reason for discarding AA is its hydrophilicity, which could result in formulation difficulties with the more lipophilic matrices.

### *5.2.2 INFLUENCE OF TEMPERATURE STRESS ON THE CONCENTRATION OF AN ETHANOLIC CBD SOLUTION*

As some derivatisations of CBD are catalysed by an increased temperature [8], it was found necessary to evaluate the stability of CBD in solutions stored at high temperatures. For this short-term study, a simple experiment was carried out (see chapter 4.3.1.2).

The experiment was made up by preparing solutions of both available CBD qualities (pure and a 94 % extract). These solutions were filled in a tightly closable glass flask and subsequently stored at 60 °C, 80 °C and 100 °C for 24 hours. The CBD content of these solutions was analysed after 1, 3 and 24 hours with HPLC.

Figure 5—11 and Figure 5—12 show the relative concentration changes of the samples during the temperature stress test. It is conspicuous that several samples showed a higher concentration after one and three hours than at the beginning. Another conspicuity is the large SD of 47.26 % for the pure CBD sample stored at 80 °C after 1440 minutes (24 h). With one other exception, every SD is  $< 7$  %. Both problems could have occurred due to an inaccurate replenishment of the samples.

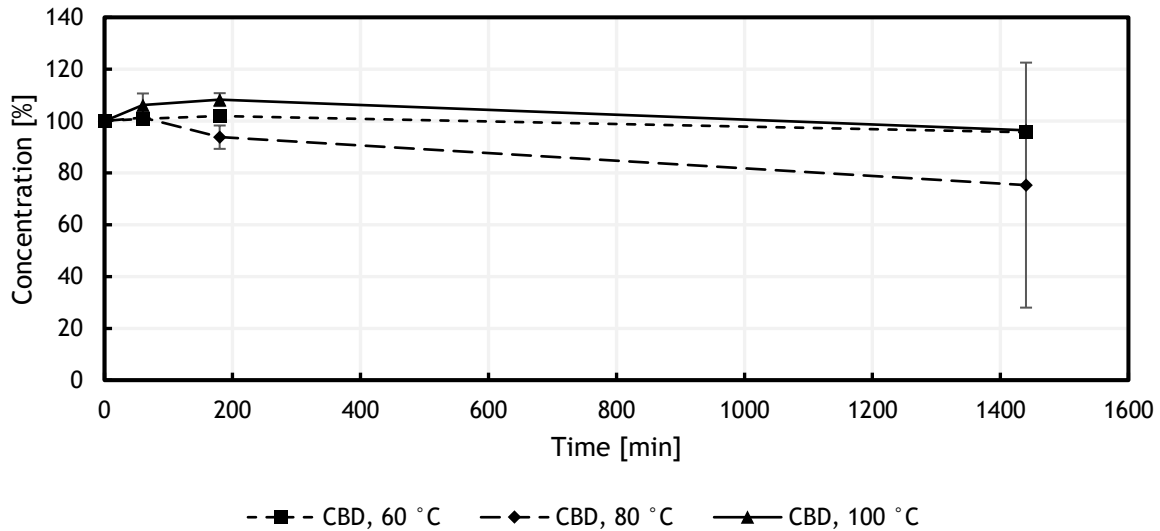


Figure 5–11: Relative CBD content of pure CBD solutions during temperature stress;  $n = 3$ ; error bars =  $SD$

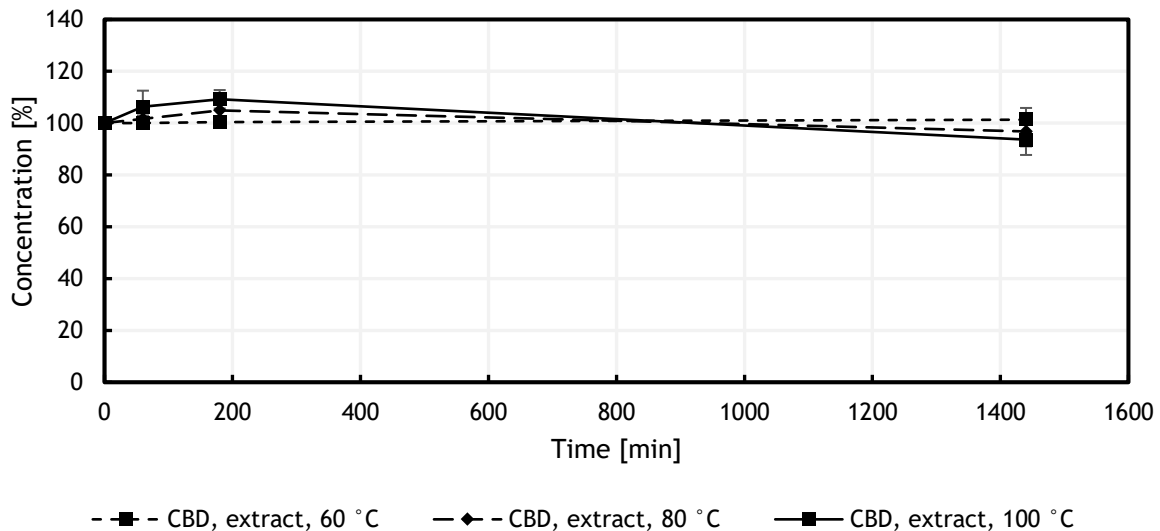


Figure 5–12: Relative CBD content of CBD extract solutions during temperature stress;  $n = 3$ ; error bars =  $SD$

After 24 h, three samples (pure: 60 °C, 100 °C; extract: 100 °C) had significantly ( $p < 0.043$ ) lower CBD concentrations. This suggests that CBD shows temperature instability, when in solution. Which indicates that formulations with CBD should not be exposed to high temperatures as the drug could degrade. This is relevant for the production of solid dispersions and strengthens the decision to prepare these with a thermally gentle process like spray drying. Due to the questionable progression of the data, it cannot be concluded if the secondary components of the extract are exercising stabilising effects on the CBD.

Concluding, the validity of these experiments must be questioned, as, in addition to the mentioned conspicuities, the 80 °C extract sample (3 h) and the 100 °C pure sample (3 h) show significantly ( $p < 0.039$ ) higher CBD concentrations than in the beginning.

## Short Term CBD Stability Stress Tests

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This experiment was not redone, although the results are obviously flawed. The reason for this is that the experimental build-up needs major changes to gain the ability to produce meaningful results. This could be achieved by implementing an internal standard. However, it would require an intensive screening procedure to identify a suitable substance. Firstly, the standard substance needs to be stable at the applied temperatures and must not be vapour-volatile. Additionally it needs to be soluble in EtOH in detectable quantities while, ideally, also being quantifiable with the used HPLC method.

### 5.3 HPMC MATRIX PARTICLES

The following chapter covers the development and production of the solid dispersions, which use HPMC as the matrix polymer. The characteristics of the two resulting formulations (with and without AP) are also included.

#### 5.3.1 DETERMINATION OF OPTIMAL SPRAY-DRYING PARAMETERS

The formulation of a solid dispersion was determined as a promising approach to formulate an inhalable CBD dry powder. HPMC (Methocel™ E5 Premium LV) was chosen as one of the matrices to incorporate CBD in a solid dispersion. It was decided to produce these with a spray-drying procedure, as this is a thermally gentle operation, which is necessary as prior experiments suggested that CBD (solutions) show an instability at increased temperatures.

To conserve CBD, it was decided to optimise the spray drying parameters for HPMC beforehand by implementing a DoE. The goal of this optimisation was the maximisation of the production yield while generating particles in an inhalable size range (0.5 – 5 µm). Three different parameters were varied (inlet temperature, aspirator rate and solid content of the spraying solution) in three different levels (Table 5–4). This resulted in a 3<sup>3</sup> full factorial design, which analysed every possible parameter combination. To assess the reproducibility the centre point was analysed three additional times, which means that this DoE had 30 total runs (Table 10–2).

Table 5–4: Variable parameters and levels for HPMC DoE

<i>Parameter</i>	<i>Level 1</i>	<i>Level 2</i>	<i>Level 3</i>
<i>Inlet Temperature [°C]</i>	100	110	120
<i>Aspirator rate [m<sup>3</sup>/h]</i>	17.5 (50 %)	26.25 (75 %)	35 (100 %)
<i>Solid Content [%]</i>	1	2	5

The resulting model for the particle size shows that only the solid content exhibits a significant influence. This was expected, as the particles are formed by precipitation, which is triggered when the solubility product is exceeded. This happens when a certain amount of solvent is evaporated, the remaining solvent volume (droplet size) defines the particle size. The model for the yield shows that both the aspirator rate and the solid content have a significant influence. Again, this was expected, as a higher aspirator rate results in a higher airflow velocity, which in turn increases the separation of smaller particles from the airflow due to higher centrifugal forces in the cyclone. The higher solid content results in a higher particle size, which increases the separation from the air stream in the cyclone. These findings are

also consistent with equipment information provided by Büchi [143]. However, the contour plot for the yield (Figure 5–13 left) shows that a decreasing inlet temperature seems to marginally increase the yield. This is unexpected and oppositional to Büchis' equipment information. Here, It is suspected that a higher inlet temperature yields a dryer product which reduces sticking to the inner surfaces of the spray dryer [144]. The influences of increasing a respective parameter are depicted in Table 5–5.

Table 5–5: Influence of parameter increase on the critical outcomes; \* signals significance

Outcome	Inlet Temperature ↑	Aspirator Rate ↑	Solid Content ↑
Yield	↘	↑*	↑*
Particle Size	→	→	↑*

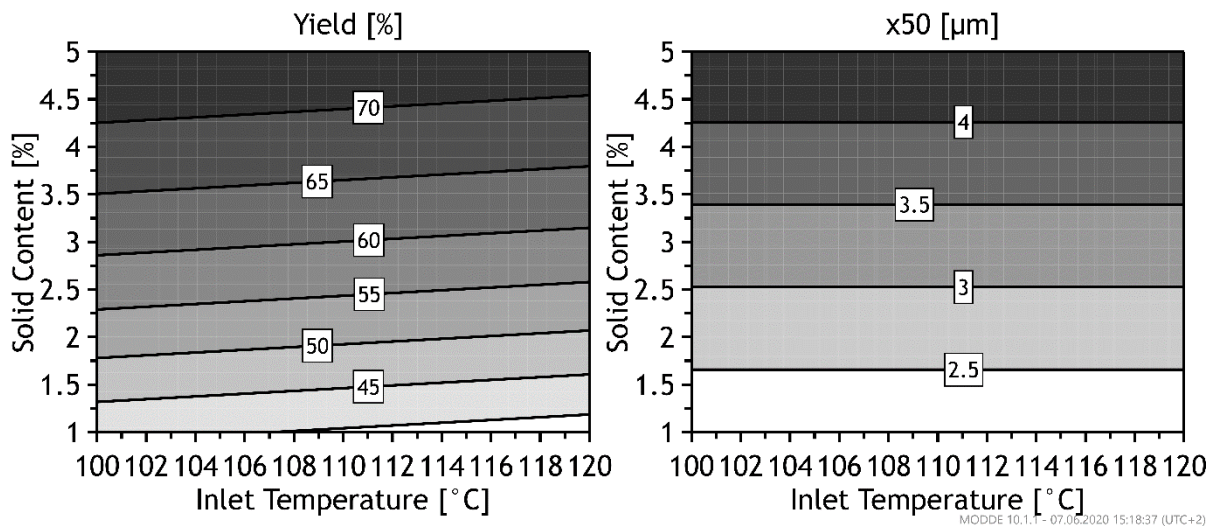


Figure 5–13: Response contour plots (left - yield; right - particle size) at 100 % aspirator setting

Only the model for the particle size has good model fit ( $R^2 = 0.92$ ) and is estimated to precisely predict future data ( $Q^2 = 0.91$ ). Model validity (0.70) and reproducibility (0.96) are also good. The values for the model of the yield show that this model is not as good ( $R^2 = 0.48$ ,  $Q^2 = 0.33$ , validity = 0.28, reproducibility = 0.92) as both  $R^2$  and  $Q^2$  are below the values which the software states as critical thresholds (both 0.5), and the validity is just above 0.25. According to the software, a model with a validity below 0.25 has significant statistical problems (e.g. outliers).

Although there are problems with the model for the yield, three different parameter settings (Table 5–6) were chosen to evaluate how efficiently CBD can be incorporated into the matrix. The settings from experiment N25 (Table 10–3) would be the optimal settings according to the generated models.



Table 5–6: Chosen parameter settings for API incorporation

<i>Parameter</i>	<i>Settings N13</i>	<i>Settings N20</i>	<i>Settings N25</i>
<i>Inlet Temperature [°C]</i>	100	110	100
<i>Aspirator rate [m<sup>3</sup>/h]</i>	35	35	35
<i>Solid Content [%]</i>	2	1	5

For the API incorporation 25 % of the matrix substance were substituted with the available CBD extract (pure CBD was not available at this point). Table 5–7 shows that the incorporation of the CBD extract reduces both the particle size and the yield of the resulting formulation. The reduced yield could be a result of the lower particle size. However, as the process resulted in a sticky drying chamber, it is also possible that a considerable amount of formulation adhered to the inner surface of the drying chamber thus reducing the yield. A subsequent DoE to reduce the sticking to the drying chamber was not carried out due to the limited amount of model drug.

The loading efficiency for each run was excellent, as 95 - 103 % of theoretically incorporated API were recovered during analysis.

Table 5–7: Influence of CBD incorporation on yield and particle size for HPMC particles

<i>Parameter</i>	<i>Settings N13</i>	<i>Settings N20</i>	<i>Settings N25</i>
<i>Particle Size without CBD [μm]</i>	2.99	2.38	4.49
<i>Particle Size with CBD [μm]</i>	1.15	1.67	2.76
<i>Yield without CBD [%]</i>	66.78	64.98	75.15
<i>Yield with CBD [%]</i>	21.63	15.72	39.01
<i>Loading Efficiency [%]</i>	95.69	102.45	99.56

As only N25 had an acceptable yield after incorporating CBD, it was concluded that these settings are optimal for the envisioned CBD formulation.

CBD was not stable in the produced formulation (N25), as the CBD content dropped from 25 % to 18 % over the course of 5 months. This was not observed during a designated experiment but randomly after storage at environmental conditions.

This preliminary formulation was also evaluated for its DD and characterised aerodynamically with the NGI (Cyclohaler®; 10 ± 0.5 mg of formulation per capsule). It achieved a mean DD of 1405 μg ± 5.4 % (recovery 87.7 ± 5.6 %), FPD of 755.2 ± 15.8 μg, a mean FPF of

54.9 ± 2.9 % and a mean MMAD of 2.7 ± 0.2 µm. It must be noted that these analyses were carried out with 85:15 acetonitrile:aq. bidest. Additionally, the formulation had a remaining CBD content of 17 % on the day of the analyses.

### 5.3.2 PRODUCTION & CHARACTERISTICS OF THE HPMC SOLID DISPERSIONS

The formulations were spray-dried as described in chapter 4.1.1. Differing from experiments described in 5.3.1, pure CBD was available for the formulation of the samples for the stability study. For the basic formulation (AO-free) 20.00 g of solid were weighed into 400 mL of EtOH (80 wt%), while 20.02 g were weighed in for the formulation with the added AO. After spray-drying, 13.31 g (basic) and 13.69 g (AO) of formulation could be recovered from the product vessel, which means that the operations achieved a yield of 66.4 % and 68.4 %, respectively. Both are distinctively higher than the 39.01 % that were observed when the CBD extract was used. This suggests that AP does not exhibit a distinct influence on the spray-drying operation, while the unknown 6 % of components of the extract exercise a strong influence on the process and thereby the resulting particles. This suggested by the resulting particle size, as both formulations have a larger  $x_{50}$  than the test batch, where the extract was used.

The particles of both formulations show the shape of a collapsed sphere (Figure 5–14) and exhibit a relatively broad PSD, which is confirmed by laser diffraction analyses. The basic formulation is slightly larger than the formulation with the AO, which could be a result of the higher amount of matrix substance.

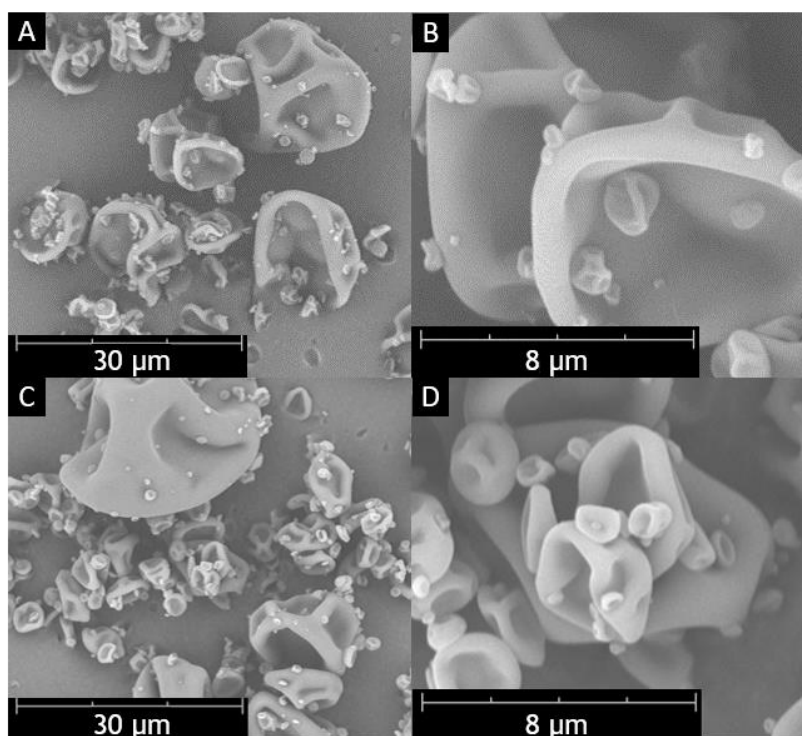


Figure 5–14: SEM images of HPMC formulations (A, B – basic formulation; C, D – AO formulation) (A, C – 2500x; B, D – 10000x)

DSC (Figure 5–15) and XRPD (Figure 5–16) results for both formulations showed no indications of crystallinity. There are no thermal events in DSC and XRPD showed a full halo for both formulations, indicating that both formulations are completely amorphous.

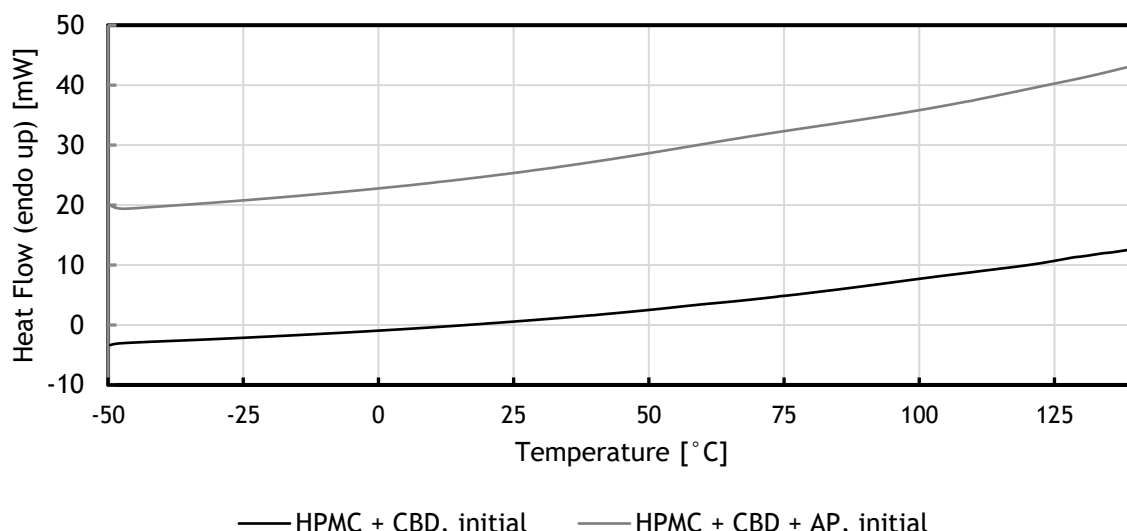


Figure 5–15: DSC heating curves of the HPMC formulations after production; AO formulation shifted by +20 mW

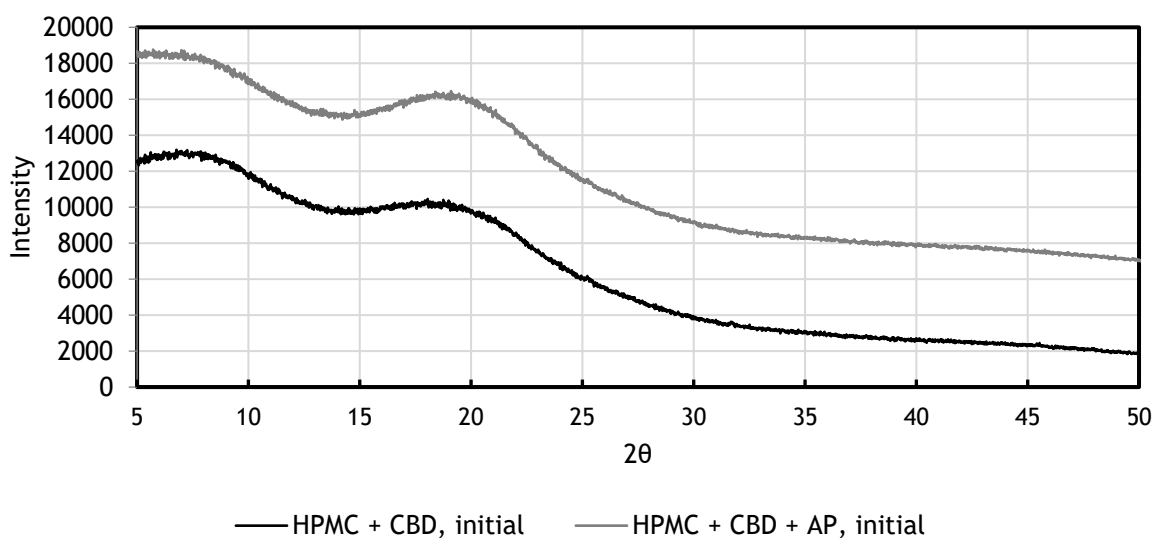


Figure 5–16: X-Ray diffractogram of HPMC formulations; after production AO formulation shifted by +5000 intensity

Table 5–8 displays the product characteristics of the HPMC formulations after production. According to the Ph.Eur. [131] both formulations are hygroscopic, as their maximum uptakes at 25 °C and 80 % rH are 10.2 % (basic) and 8.0 % (AO). The pure polymer had a distinctly higher water uptake of 24.5 %. The lower uptake of the formulations was expected, as 10 % and > 20 % of the polymer is substituted with substances with very low water. Both processes yielded excellent loading efficiencies (> 98 %), showing that CBD can also be efficiently incorporated into the matrix when the pure substance is used.

The density of both formulations is relatively close to the density of the pure, unprocessed polymer ( $1.278 \pm 0.002 \text{ g/cm}^3$ ). CBD ( $1.068 \pm 0.003 \text{ g/cm}^3$ ) and AP ( $1.182 \pm 0.009 \text{ g/cm}^3$ ) have lower densities than the polymer. This is probably the reason that the formulations have a density between the polymer and the other two substances.

Table 5–8: Initial product characteristics of the HPMC formulations (left – basic formulation; right – AO formulation);  $n = 3$ ;  $n = 10$ ; *cursive: n = 1*

<i>HPMC + CBD</i>	<i>Result</i>	<i>SD</i>	<i>HPMC + CBD + AP</i>	<i>Result</i>	<i>SD</i>
<i>Density [g/cm<sup>3</sup>]</i>	<u>1.261</u>	<u>0.015</u>	<i>Density [g/cm<sup>3</sup>]</i>	<u>1.229</u>	<u>0.006</u>
<i>Particle Size (x<sub>50</sub>) [μm]</i>	6.84	0.20	<i>Particle Size (x<sub>50</sub>) [μm]</i>	6.36	0.22
<i>Span</i>	2.73	0.07	<i>Span</i>	2.53	0.08
<i>Maximum Water Uptake [%]</i>	16.9	N/A	<i>Maximum Water Uptake [%]</i>	13.9	N/A
<i>CBD Content [%]</i>	10.07	0.01	<i>CBD Content [%]</i>	9.84	0.00
<i>Loading Efficiency [%]</i>	100.8	0.1	<i>Loading Efficiency [%]</i>	98.1	0.0

### 5.3.3 AERODYNAMIC CHARACTERISATION

During aerodynamic characterisation, it was found out, that the formulation was only dispersible with the Cyclohaler®. Inhalation manoeuvres with the Diskus® were not able to expel the formulation from the blister cup (Figure 5–17). This was the case for both HPMC formulations as well as the HPMC-AS and Soluplus® formulations in the following chapters. Therefore, all following aerodynamic data were generated solely with the Cyclohaler®.

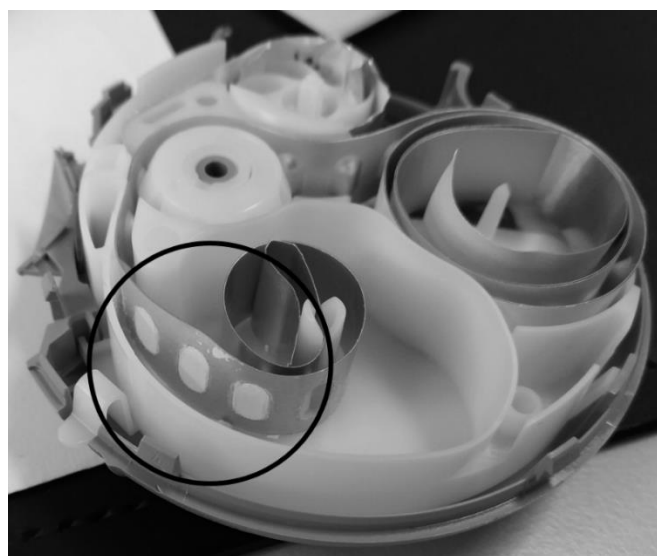


Figure 5–17: Diskus® after simulated inhalation manoeuvre; encircled area shows full blister cups after inhalation manoeuvres

The three respective DUSA analyses showed a mean DD of  $971.1 \mu\text{g} \pm 11.2 \%$  for the basic formulation and  $846.6 \mu\text{g} \pm 28.2\%$  for the formulation with AP. It should be mentioned that these analyses had relatively low and irregular recoveries of  $66.7 \pm 9.2 \%$  (basic) and  $62.6 \pm 16.6 \%$  (with AP). The low recovery may be a result of insufficient rinsing time of the components or a less than optimal solvent. The solvent for the quantification during the stability study, however, could not be used for this analysis, as acetonitrile dissolves the Cyclohaler<sup>®</sup>. It has to be kept in mind that DD analyses were done in triplicate and not ten times, which is divergent from the Ph. Eur. [24]. This distinctly reduces the explanatory power of these analyses. Therefore, these values have to be seen as an approximation.

The comparison of the absolute DD of two measurements with distinctly different recoveries comprises the risk of wrongly considering a formulation as inferior. Therefore, the adjusted DD was implemented, which analyses the DD based on the recovery instead of the theoretical API content of the capsule (Equation (4–2)). However, it has to be kept in mind that this type of data evaluation is no substitution for measurements with adequate recoveries.

In this case, it is emanated that the low recovery is a result of an incomplete dissolution of the formulations, which is a result of a reduced dissolution velocity in the alternative solvent (MeOH:Aq. Bidest.), which had to be used due to the susceptibility of the device to acetonitrile. This problem could maybe have been avoided by using a higher amount of solvent. However, every DD analyses showed that the highest amount of formulation was found either in the tube or the combination of mouthpiece and filter. This shows that it is unlikely that the formulation that was not emitted from the device or capsule was incompletely dissolved. Therefore, it can assumed that the calculation of the adjusted DD will undervalue the true DD of the formulations. However, in a lesser extent than when the total API is used for the calculation, while achieving a distinctly higher comparability of the formulations.

The adjusted DD for the basic formulation was  $94.7 \pm 2.7 \%$  and the formulation with AP achieved an adjusted DD of  $90.2 \pm 3.6 \%$ , which are respectable values. Regarding the adjusted DD, it has to be kept in mind that the analysis on the basis on differing recoveries comprises the risk of enhancing errors. However, as the absolute DD of two measurements with distinctly differences is not comparable, the adjusted DD was seen as an acceptable alternative.

Table 5–9 shows the mean results of the NGI analyses. It is conspicuous that the recovery of the NGI analysis is much higher while the SD is a lot smaller than for the DUSA analysis, this could be due to the much higher amount of total solvent used during the analyses (105 mL to 45 mL), which could result in a more thorough dissolution depending on the deposition profile. It must also be kept in mind that, due to the susceptibility of the inhaler to acetonitrile, a less than optimal solvent had to be used.

The considerably lower MMAD (compared to the geometric diameter) shows that the particles have a relatively high dynamic shape factor, as the density of the formulations is higher than 1 g/cm<sup>3</sup> (see Table 5–8).

Table 5–9: NGI results of the HPMC formulations (left – basic formulation; right – AO formulation); n = 3; Cyclohaler®

HPMC + CBD	Result	SD	HPMC + CBD + AP	Result	SD
FPD [ $\mu\text{g}$ ]	283.2	13.0	FPD [ $\mu\text{g}$ ]	251.0	8.4
FPF [%]	24.6	1.9	FPF [%]	22.1	1.1
MMAD [ $\mu\text{m}$ ]	3.9	0.5	MMAD [ $\mu\text{m}$ ]	4.6	0.5
Recovery [%]	79.4	3.5	Recovery [%]	81.1	6.6

Figure 5–18 shows the mean deposition profile of both formulations. Most of both formulations impacts between the throat and stage 1. These three stages also have the highest SDs. Furthermore, it can be seen, that less of the formulations impacts at every successive stage. However, it is also visible that most of the formulation leaves the device, which confirms the findings of dose uniformity analyses. The formulations show a comparable deposition profile, indicating that the addition of AP has no large influence on the aerodynamic behaviour of the formulation.

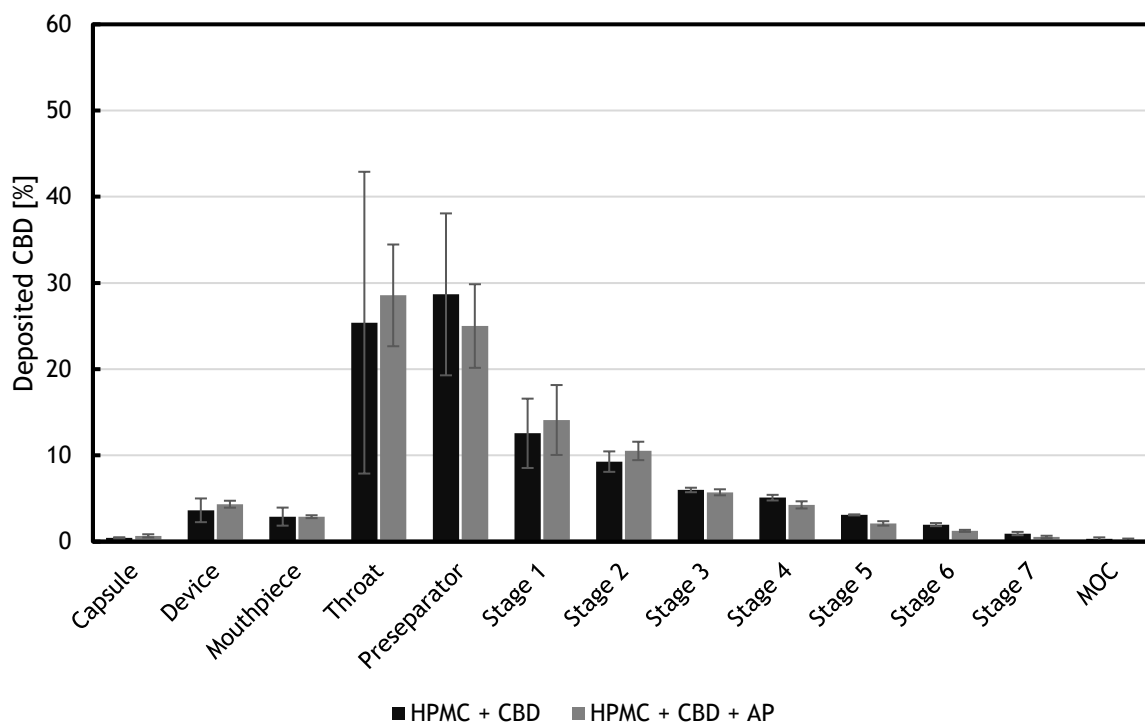


Figure 5–18: Initial mean deposition profile (NGI) of the HPMC formulations; n = 3; error bars = SD; Cyclohaler®

When both formulations are compared most parameters are statistically similar. The basic formulation, however, had higher FPD ( $p = 0.023$ ) and FPF values ( $p = 0.123$ ). This suggests that the basic formulation has a slightly superior aerodynamic performance.

The results are inferior to those of the preliminary formulation with 25 % CBD content (extract). This is probably a result of the considerably higher geometrical particle size ( $6.84 \mu\text{m}$  vs.  $2.76 \mu\text{m}$ ), although it could be possible that the preliminary particles had a different dispersion behaviour due to different cohesion forces. This could be a result of the extract, which influenced the spray-drying process considerably.

Table 5–10 shows the cut-off values, which are, according to chapter 2.9.18 of the Ph. Eur. [134], corresponding to the used airflow. This explains that most of the particles which can be viewed as inhalable (see chapter 2.2) impact between stage 2 and 6.

Table 5–10: Cut-off values (MMAD) of the individual stages in the NGI at the defined measuring conditions of 100 L/min

Stage	1	2	3	4	5	6	7	MOC
Lower Cut-off Value (MMAD) [ $\mu\text{m}$ ]	8.06	4.46	2.82	1.66	0.94	0.55	0.34	0

Due to the low recoveries, observed during DUSA analyses, two measurements of the formulation with AP did not meet the specifications of the Ph. Eur. (see chapter 4.2.7.2), as they delivered 131.3 % and 144.9 % of the mean DD values.

## 5.4 HPMC-AS MATRIX PARTICLES

The following chapters cover the development and production of the solid dispersions which use HPMC-AS as the matrix polymer. This polymer was chosen due to its increased lipophilicity and its effectiveness as a solid dispersion carrier. It is expected that this polymer yields smaller particles due to its higher solubility in organic solvents.

The characteristics of the two resulting formulations (with and without AP) are also included.

### 5.4.1 DETERMINATION OF OPTIMAL SPRAY-DRYING PARAMETERS

Like with the HPMC matrix, it was chosen to optimise the spray-drying parameters before incorporating the model drug, to conserve substance. The optimisation, again, focussed on maximising the yield while maintaining an inhalable particle size of 0.5 – 5 µm.

As the experiments with HPMC showed that a maximum aspirator setting increases the yield, which is consistent with equipment information provided by Büchi [143]. The aspirator setting was fixed to 100 %. The inlet temperature and the solid content of the spraying solution were chosen as the variable parameters with three different levels (Table 5–11). Additionally, the HPMC-AS quality was introduced as a qualitative factor (LF and MF).

For this parameter combination, the Modde software recommended the implementation of an L18 DoE. An L18 design is a so called fractional factorial design, which uses one (often qualitative) factor at two levels and up to seven factors at three levels. This resulted in a total of 21 runs, as a single run was repeated three times as a fictional centre point (Table 10–4).

Table 5–11: Variable parameters and levels for HPMC-AS DoE

<i>Parameter</i>	<i>Level 1</i>	<i>Level 2</i>	<i>Level 3</i>
<i>Inlet Temperature [°C]</i>	70	100	130
<i>Solid Content [%]</i>	1	2	5
<i>HPMC-AS Type</i>	LF		MF

Alike HPMC, the yield and the particle size, represented by the  $x_{50}$  value, were viewed as the critical outcomes. The resulting model identified both the inlet temperature as well as the solid content and the product of these factors as significant on the yield. A lower inlet temperature and a higher solid content was found to result in better yields (left side of Figure 5–19 & Figure 5–20). The particle size is only significantly altered by the solid content. Here a higher solid content will result in larger particles (right side of Figure 5–19 & Figure 5–20). Due to the qualitative factor, it was not possible to repeat a true centre point. This results in



an inability to evaluate the model fit and the reproducibility without repeating the whole set of experiments. This was not done, due to limited quantities of the matrix polymers.

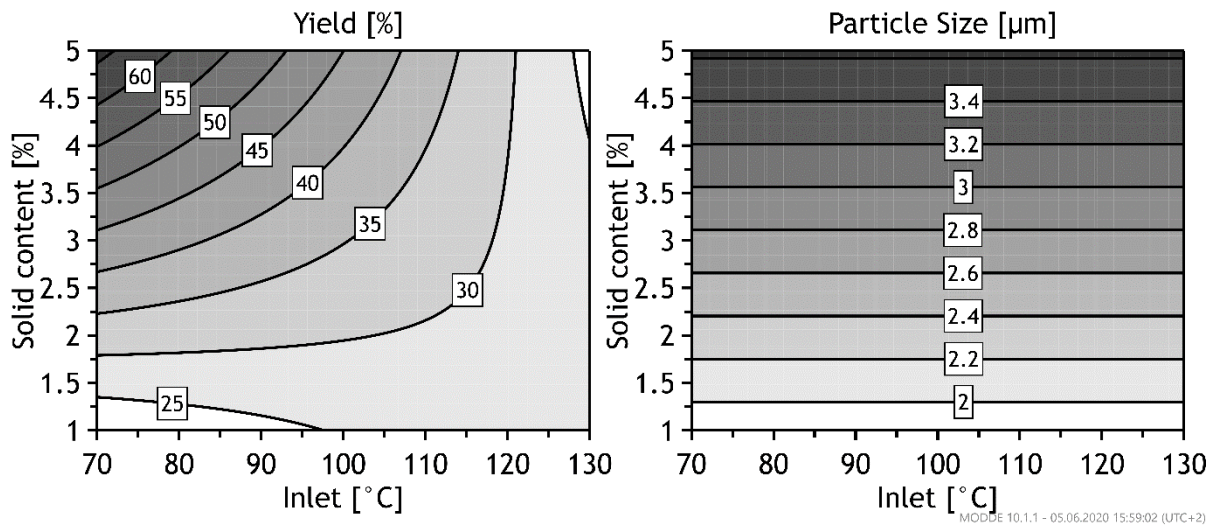


Figure 5–19: Response contour plots (left - yield, right - particle size) HPMC-AS MF

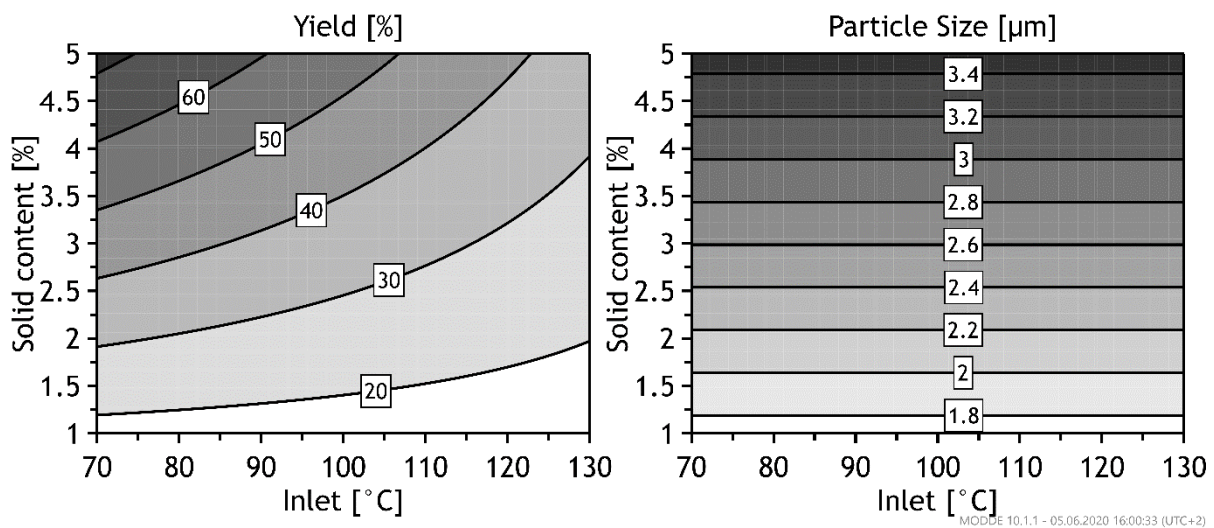


Figure 5–20: Response contour plots (left - yield, right - particle size) HPMC-AS LF

A frequent problem during the spray-drying experiments was that the cyclone tended to clog with powder. The incorporation of CBD (extract) resulted in a decreasing particle size for the HPMC particles. Therefore, the MF type was chosen for further experiments as the model predicts larger particles for this type, although the model suggests that the LF quality is more efficient in producing higher yields. According to the generated model the optimal spray-drying parameters for the chosen HPMC-AS type are at 70 °C and with 5 % solid content in the spraying solution.

The incorporation of 25 % of CBD extract (pure CBD was not yet available) reduced both the yield (53.94 % to 13.31 %) and the particle size (3.49 µm to 2.27 µm). The loading efficiency,

however, was excellent with about 105 %. The value above 100 % could be a result of measuring inaccuracies, an inhomogeneous distribution in the formulation or a loss matrix during the spray-drying procedure or incomplete dissolution. Therefore, it was decided that the approach of using HPMC-AS as a matrix for CBD is promising and should be included in the stability study.

It was observed that CBD is not stable in the produced formulation, as the CBD content dropped from 26.27 % to 20 % over the course of 1.5 months. Like with the HPMC particles, this was not evaluated in a designated experiment.

### *5.4.2 PRODUCTION & CHARACTERISTICS OF THE HPMC-AS SOLID DISPERSIONS*

This formulation was produced via spray-drying with the parameters defined in chapter 4.1.1. For both formulations, 20.01 g of solids were weighed into the spraying solution. This resulted in 14.96 g (basic formulation) and 12.11 g (AO formulation) of product. This corresponds with yields of 74.8 % and 60.6 %. As observed with the HPMC formulations, the utilisation of pure CBD, instead of the extract, lead to distinctively higher yields, as the one achieved subsequent to the determination of the optimal spray-drying parameters. It can also be assumed, that the replacement of matrix substance with AP has an influence on the production process of HPMC-AS particles, as the yield with the AO is considerably lower. This is probably due to the later precipitation (from smaller droplets) of the matrix during the process, which results in smaller particles and thus an increased loss of formulation. This is a result of the increased probability of smaller particles to be carried to the filter of the spray dryer.

The particles of the HPMC-AS formulations have similar shapes as the HPMC particles: A collapsed sphere (Figure 5–21). Again, broad PSDs are observable and confirmed by laser diffraction analyses (Table 5–12). The  $x_{50}$  of the basic formulation is slightly larger than that of the formulation with AP, which is probably due to the similar reasons as the HPMC formulations. As expected (see chapter 5.4), these formulations have a smaller  $x_{50}$  than the HPMC formulations. This could be a result of the higher lipophilicity of HPMC-AS, which in turn leads to a later precipitation in the solvent during the spray-drying procedure. However, the SD of the basic formulation is very high, which shows that this might be a measuring inaccuracy.

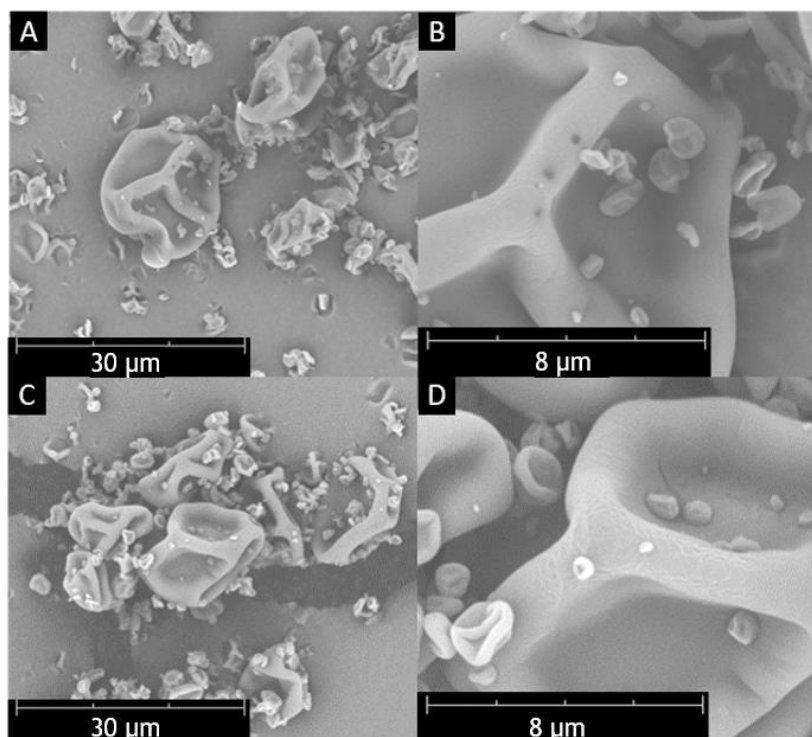


Figure 5—21: SEM images of HPMC-AS formulations (A, B - basic formulation; C, D - AO formulation) (A, C - 2500x; B, D - 10000x)

Similar to the HPMC formulations, the HPMC-AS formulations are completely amorphous, as both DSC and XRPD show no indications of crystallinity. The diagrams are omitted due to similar curves as those depicted in Figure 5—15 and Figure 5—16.

Table 5—12 displays the initial product characteristics. Like the HPMC formulations, these formulations show hygroscopic behaviour, according to the Ph.Eur. [131], with a maximum uptake of 5.6 % (basic formulation) and 4.7 % (AO formulation) at 25 °C and 80 % rH. The pure polymer has a water uptake of 10.3 %. It was expected that the water uptake of the formulations would be lower than that of the pure polymer, as 10 % and > 20 % of the polymer is substituted with substances with very low water uptakes.

Both formulations exhibit a density that is slightly above that of the unprocessed polymer ( $1.204 \pm 0.008 \text{ g/m}^3$ ). CBD ( $1.068 \pm 0.003 \text{ g/m}^3$ ) and AP ( $1.182 \pm 0.009 \text{ g/m}^3$ ) have even lower densities than the polymer. Therefore, it is unexpected that the density of the formulation is higher than that of the raw substances. The reason for the slightly higher density of the products could be the relatively small sample size, due to the small batch sizes, in the pycnometer. It is possible that the measured particle collective had a higher mean density than that of the formulation in total.

## HPMC-AS Matrix Particles

Table 5—12: Initial product characteristics of the HPMC-AS formulations (left – basic formulation; right – AO formulation);  $n = 3$ ;  $n = 10$ ; *cursive*:  $n = 1$

<i>HPMC-AS + CBD</i>	<i>Result</i>	<i>SD</i>	<i>HPMC-AS + CBD + AP</i>	<i>Result</i>	<i>SD</i>
<i>Density [g/cm<sup>3</sup>]</i>	<u>1.231</u>	<u>0.009</u>	<i>Density [g/cm<sup>3</sup>]</i>	<u>1.228</u>	<u>0.006</u>
<i>Particle Size (x<sub>50</sub>) [μm]</i>	5.00	0.27	<i>Particle Size (x<sub>50</sub>) [μm]</i>	4.48	0.07
<i>Span</i>	3.27	0.05	<i>Span</i>	2.94	0.02
<i>Maximum Water Uptake [%]</i>	7.2	N/A	<i>Maximum Water Uptake [%]</i>	6.2	N/A
<i>CBD Content [%]</i>	9.88	0.00	<i>CBD Content [%]</i>	9.61	0.01
<i>Loading Efficiency [%]</i>	98.6	0.0	<i>Loading Efficiency [%]</i>	96.1	0.1

### 5.4.3 AERODYNAMIC CHARACTERISATION

The three respective dose uniformity analyses showed mean DDs of  $985.6 \mu\text{g} \pm 22.4 \%$  (basic formulations) and  $698.4 \mu\text{g} \pm 21.7 \%$ . Alike the HPMC formulations, the recoveries for the HPMC-AS formulations were relatively low and irregular  $72.4 \pm 12.6 \%$  (basic formulation) and  $56.2 \pm 10.1 \%$  (AP formulation). However, the adjusted DDs for these formulations are respectable, with values of  $90.6 \pm 4.3 \%$  (basic formulation) and  $85.5 \pm 2.2 \%$  (AO formulation).

Table 5—13 shows the mean results of the NGI analyses of the HPMC-AS formulations. Of the six formulations (all three matrices), the basic HPMC-AS formulation had the best recovery, while the formulation with AP had the lowest recovery during the initial aerodynamic characterisation. The observations about the MMADs are consistent with the HPMC formulations.

Table 5—13: NGI results of the HPMC-AS + CBD formulation

<i>HPMC + CBD</i>	<i>Result</i>	<i>SD</i>	<i>HPMC + CBD + AP</i>	<i>Result</i>	<i>SD</i>
<i>FPD [μg]</i>	400.4	25.7	<i>FPD [μg]</i>	288.0	13.6
<i>FPF [%]</i>	29.4	1.6	<i>FPF [%]</i>	27.9	1.1
<i>MMAD [μm]</i>	3.5	0.3	<i>MMAD [μm]</i>	3.7	0.2
<i>Recovery [%]</i>	93.7	0.8	<i>Recovery [%]</i>	75.5	2.1

Figure 5—22 shows the mean deposition profiles of the HPMC-AS formulations. As observed with the HPMC formulations, most of these formulations is emitted from the device and impacts between the throat and stage 1. However, the SDs observed in these analyses are distinctively smaller than those of the former formulations. Higher amounts of these formulation

reach the latter stages. This is confirmed by the determined FPDs and FPFs, as the HPMC-AS formulations achieved the best values in both parameters.

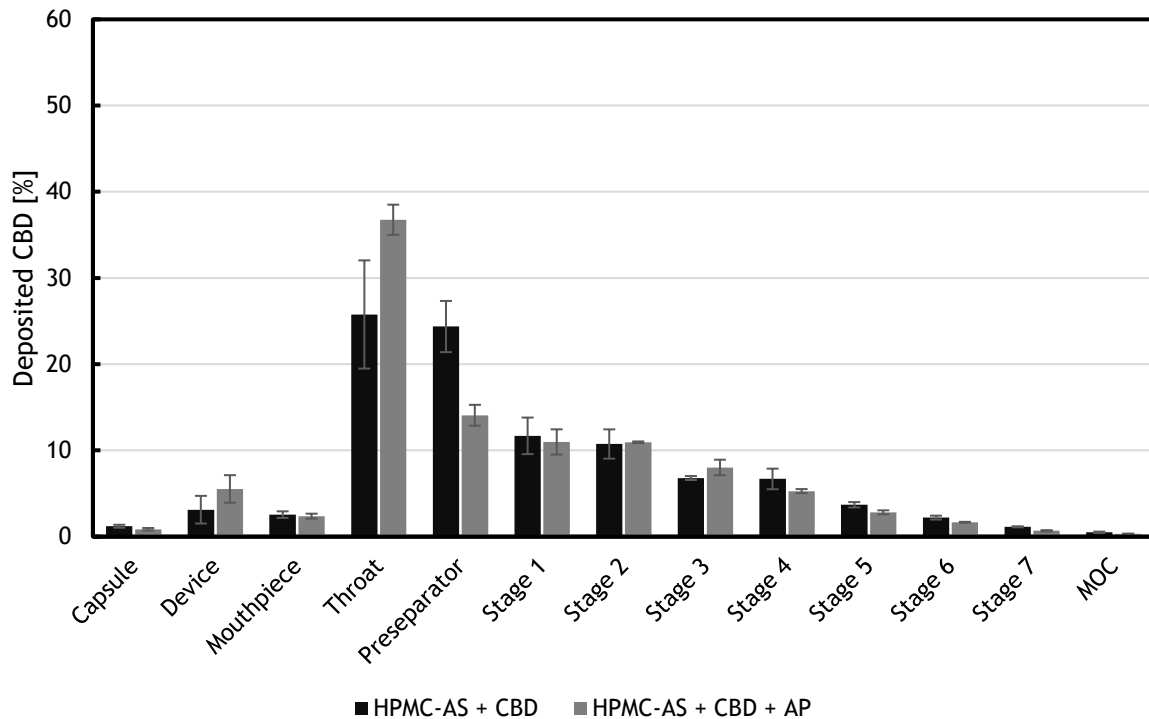


Figure 5–22: Initial mean deposition profile (NGI) of the HPMC-AS formulations;  $n = 3$ ; error bars = SD; Cyclohaler®

Like the HPMC formulations, the basic formulation seems to be a slightly superior from an aerodynamic standpoint, as the FPD ( $p = 0.003$ ) and FPF values ( $p = 0.245$ ) are larger.

No run of both formulations met the specifications of the Ph. Eur. (see chapter 4.2.7.2). The lowest recovery (compared to the DD analyses) for the basic formulation was 135.1%, whereas each run of the AP formulation had a recovery of at least 144.9%. This, again, is a result of the different recoveries of the DD and NGI analyses, which probably is a result of the less than optimal solvent as well as the increased solvent volume that is used during NGI analyses.

## 5.5 SOLUPLUS® MATRIX PARTICLES

The following chapters cover the production of the solid dispersions which use SP as the matrix polymer. The characteristics of the two resulting formulations (with and without AP) are also included.

### 5.5.1 PRODUCTION & CHARACTERISTICS OF THE SOLUPLUS® SOLID DISPERSIONS

Like the formulations that utilise one of the other two polymers, the production parameters for these formulations are defined in chapter 4.1.1. 20.02 g of solids were weighed into the spraying solution for both formulations, resulting in 13.13 g (basic formulation; yield: 66.57 %) and 11.84 g (AO formulation; yield: 59.16 %) of product, respectively. The AO formulation had the lowest yield of the six produced formulations.

In contrast to the HPMC(-AS) particles, these formulations have more spherical particles. The particles of the basic formulation have some indentions and the formulation that contains AP has almost perfect spherical particles (Figure 5–23). Although these particles show a distinctively different shape, their PSD, like with the other four formulations, is relatively broad, which confirms the findings of laser diffraction (Table 5–14). The SP formulations are the only formulation in which the addition of AP results in larger particles. Which could be a result of the fact that the particles of the AP formulation did not collapse.

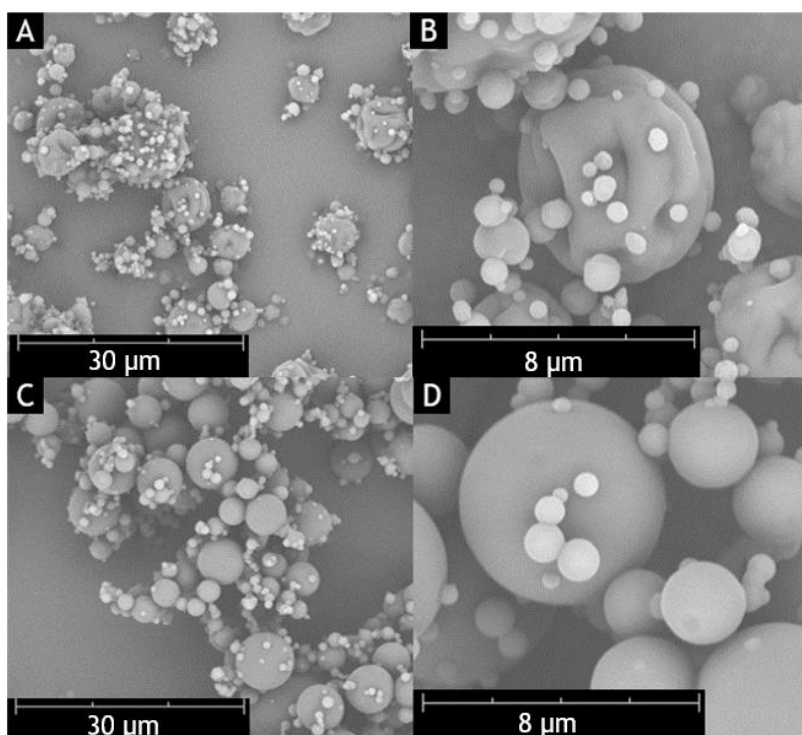


Figure 5–23: SEM images of SP formulations (A, B – basic formulation; C, D – AO formulation) (A, C – 2500x; B, D – 10000x)

Both formulations show a similar behaviour in DSC and XRPD as the formulations with the other matrices: no thermal events in DSC and a full halo in XRPD. This shows that these formulations are also completely amorphous.

Table 5–14 displays the initial product characteristics. According to the Ph.Eur. [131], both formulations are hygroscopic, as they exhibit a maximum uptake of 9.4 % (basic formulation) and 6.3 % (AO formulation) at 25 °C and 80 % rH. The pure polymer has a water uptake of 21.6 %. The lower water uptake of the formulations was expected, as 10 % and > 20 % of the polymer is substituted with substances with very low water uptakes. The relatively high water uptake of the pure polymer was also expected, as it was specifically designed for solid solutions and the solubilisation of poorly soluble substances in aqueous media.

The basic formulation shows a density that is slightly above that of the unprocessed polymer ( $1.174 \pm 0.004 \text{ g/cm}^3$ ), whereas the density of the AP formulation is distinctly higher. CBD ( $1.068 \pm 0.003 \text{ g/cm}^3$ ) has a lower density, whereas the density of AP ( $1.182 \pm 0.009 \text{ g/cm}^3$ ) is slightly higher than that of SP. However, the density of the base formulation is consistent with the expectation that the formulation exhibits a density between that of the raw substances. The formulation with AP shows a higher density than any of the used materials. This is unexpected but could be a result of the small sample size measured in the pycnometer. The analysed collective could be a cluster of particles with a higher mean density than that of the total formulation. However, measurement of more samples was not possible due to the batch size.

Table 5–14: Initial product characteristics of the SP formulations (left – basic formulation; right – AO formulation);  $n = 3$ ;  $\bar{n} = 10$ ; *cursive: n = 1*

<i>SP+ CBD</i>	<i>Result</i>	<i>SD</i>	<i>SP + CBD + AP</i>	<i>Result</i>	<i>SD</i>
<i>Density [g/cm<sup>3</sup>]</i>	<u>1.165</u>	<u>0.007</u>	<i>Density [g/cm<sup>3</sup>]</i>	<u>1.333</u>	<u>0.014</u>
<i>Particle Size (x<sub>50</sub>) [μm]</i>	5.65	0.11	<i>Particle Size (x<sub>50</sub>) [μm]</i>	6.59	0.17
<i>Span</i>	<u>2.27</u>	<u>0.04</u>	<i>Span</i>	<u>2.01</u>	<u>0.03</u>
<i>Maximum Water Uptake [%]</i>	16.5	N/A	<i>Maximum Water Uptake [%]</i>	17.0	N/A
<i>CBD Content [%]</i>	<u>9.98</u>	<u>0.01</u>	<i>CBD Content [%]</i>	<u>9.69</u>	<u>0.00</u>
<i>Loading Efficiency [%]</i>	99.6	0.1	<i>Loading Efficiency [%]</i>	96.8	0.0

### 5.5.2 AERODYNAMIC CHARACTERISATION

The three dose uniformity analyses of the basic formulation showed a mean DD of 1142.2 µg ± 9.2 % with a mean recovery of 79.3 ± 8.6 %. This yielded an adjusted DD of 95.4 ± 0.3 %. The AO formulation had a mean DD of 1116.1 µg ± 10.5 % with a recovery of 80.5 ± 5.9 % (highest mean value and lowest SD of the initial characterisations). The adjusted DD was 95.2 ± 2.4 %.

Table 5—15 shows the mean results of the NGI analyses. The discrepancies between the recoveries of the SP formulations NGI and DUSA runs is not as high as some of the previously observed recoveries. Actually, the formulation with AP is the only formulation which had a higher recovery for DUSA analyses. The basic formulation shows a slightly smaller MMAD than its geometrical diameter. The software, however, was unable to determine an MMAD for the AP formulation. This is a result from the distribution pattern (Figure 5—24) and the low FPD.

Table 5—15: NGI results of the SP + CBD formulation

<i>HPMC + CBD</i>	<i>Result</i>	<i>SD</i>	<i>HPMC + CBD + AP</i>	<i>Result</i>	<i>SD</i>
<i>FPD [µg]</i>	225.7	24.1	<i>FPD [µg]</i>	98.7	5.9
<i>FPF [%]</i>	17.4	2.9	<i>FPF [%]</i>	8.9	0.8
<i>MMAD [µm]</i>	4.5	0.1	<i>MMAD [µm]</i>	N/A	N/A
<i>Recovery [%]</i>	88.7	5.2	<i>Recovery [%]</i>	79.3	1.3

Figure 5—24 shows the mean deposition profile of the SP formulations. The basic formulation is the only one of which more than 40 % are deposited in the throat. The formulation with AP is the only one of which more than 30 % are deposited in the preseparator and the only formulation which is not depositing after stage five. It also has by far the lowest FPF and FPD values, about half the values of the second lowest formulation (SP + CBD). However, the patterns are similar to the other matrices, as every subsequent stage has less deposited formulation.



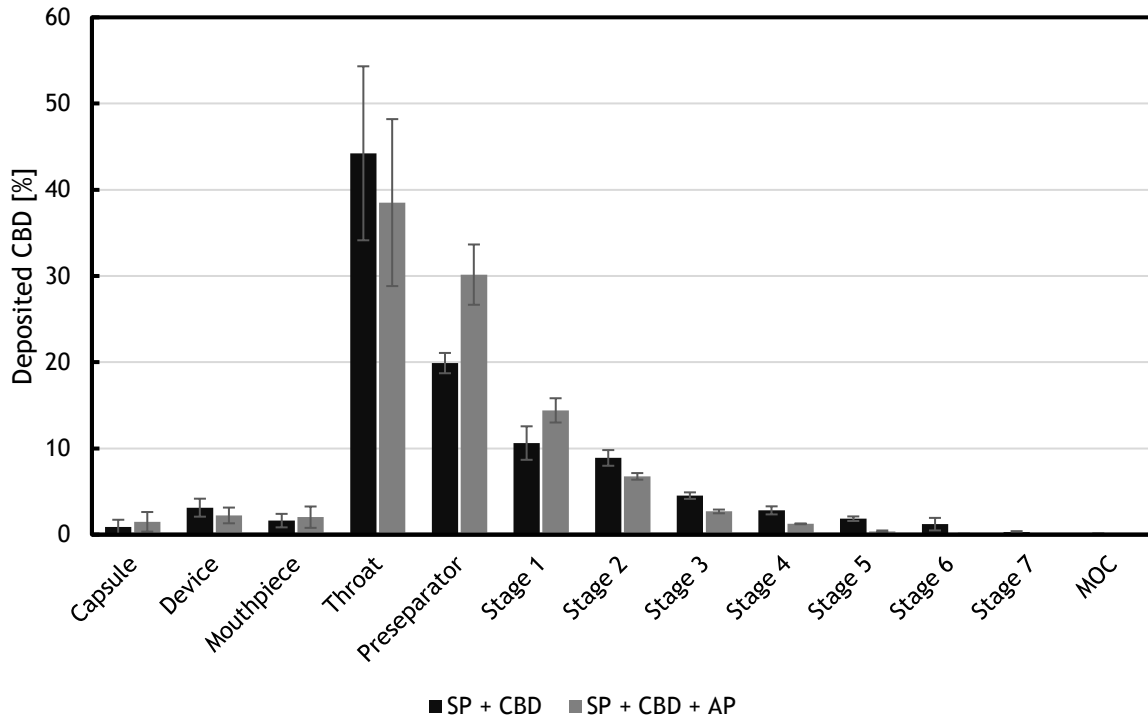


Figure 5–24: Mean deposition profile (NGI) of the SP + CBD formulation, initial;  $n = 3$ ; error bars = SD; Cyclohaler®

The basic formulation is clearly superior to the second formulation, as both the FPD and FPF are significantly higher ( $p < 0.009$ ).

Every run of both formulations met the specification of a recovery, compared to DD analyses, of 75 – 125 %.

Of the six formulations, the basic HPMC-AS formulations seem to be the most promising due to the already solid aerodynamic performance and low particle sizes. However, it is not possible to make assumptions about the stability of the API during storage in the different matrices. Due to prior experiments it can be suspected that the formulations with AP exhibit a better storage stability for the API. Regarding the susceptibility to the external storage conditions the HPMC-AS formulations should be superior, due to the increased lipophilicity and decreased hygroscopicity, as less water should be able to react with the particles.

## 5.6 TWELVE-WEEK STABILITY OF THE CBD MATRIX PARTICLES

The following chapters present the data of the conducted stability study.

The stability study was carried out to evaluate if the developed formulations are influenced or changed by the storage conditions. The most interesting parameter for this was the change of the CBD content. This enabled to evaluate if the model drug is degraded and if different storage conditions (5 °C, 25 °C & 60 % rH or 40 °C & 75 rH) packaging (bulk, capsule or blister) or the chosen AO (AP) have an influence on the degradation velocity. Additionally, the influence of the storage conditions on the physicochemical properties and the aerodynamic behaviour of the formulation was evaluated.

It is expected that the most stressful conditions (40 °C & 75 % rH) exert the highest influence on the formulations. Firstly, chemical reactions, like a degradation, are accelerated at higher temperatures. Additionally, a higher amount of relative humidity increases the amount of water which is adhered to the formulation and able to react with the substances, which could mean an increased degradation or physico-chemical reactions, like the formation of agglomerates. Consequently, the formulations should be the most stable when stored in the refrigerator.

Overall, it is expected that a single formulation can be classified as auspicious for future research and/or optimisation.

### 5.6.1 ADHERENCE TO THE DEFINED STORAGE CONDITIONS

As stated in chapter 4.3.2.1, the samples were stored under three different storage conditions (Table 4–10) which were monitored externally. Although it was not specified, the rH in the refrigerator, which was used for the 5 °C condition, was monitored. The logged temperatures are depicted in Figure 5–25 to Figure 5–27 while the rHs are shown in Figure 5–28 to Figure 5–30.

The x-axis of the diagrams shows the elapsed time in minutes. The auxiliary lines are separated by one week while the main lines are separated by four weeks.

Table 5–16: Temperature data points above/below thresholds (5 ± 3 °C; 25 ± 2 °C; 40 ± 2 °C)

<i>Storage condition</i>	<i>5 °C</i>	<i>25 °C &amp; 60 % rH</i>	<i>40 °C &amp; 75 % rH</i>
<i>Total data points</i>	4432	4437	4412
<i>Data points above upper threshold</i>	2	6	0
<i>Data points below lower threshold</i>	64	1	7

Table 5—17: Humidity data points above/below thresholds ( $60 \pm 5 \% \text{ rH}$ ;  $75 \pm 5 \% \text{ rH}$ )

<i>Storage condition</i>	<i>25 °C &amp; 60 % rH</i>	<i>40 °C &amp; 75 % rH</i>
<i>Total data points</i>	4437	4412
<i>Data points exceeding upper threshold</i>	8	0
<i>Data points undercutting lower threshold</i>	23	25

Table 5—16 and Table 5—17 show the total amount of data points for each storage condition and the number of points which are above or below the defined thresholds. Table 5—16 represents the temperature data and Table 5—17 shows the humidity data.

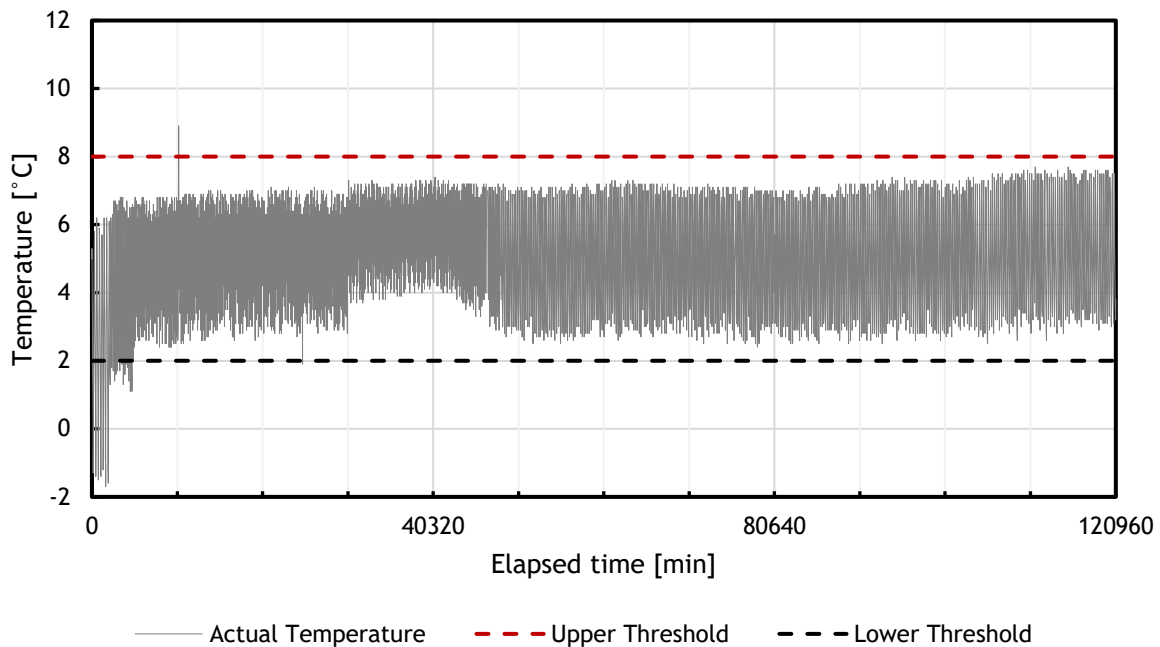
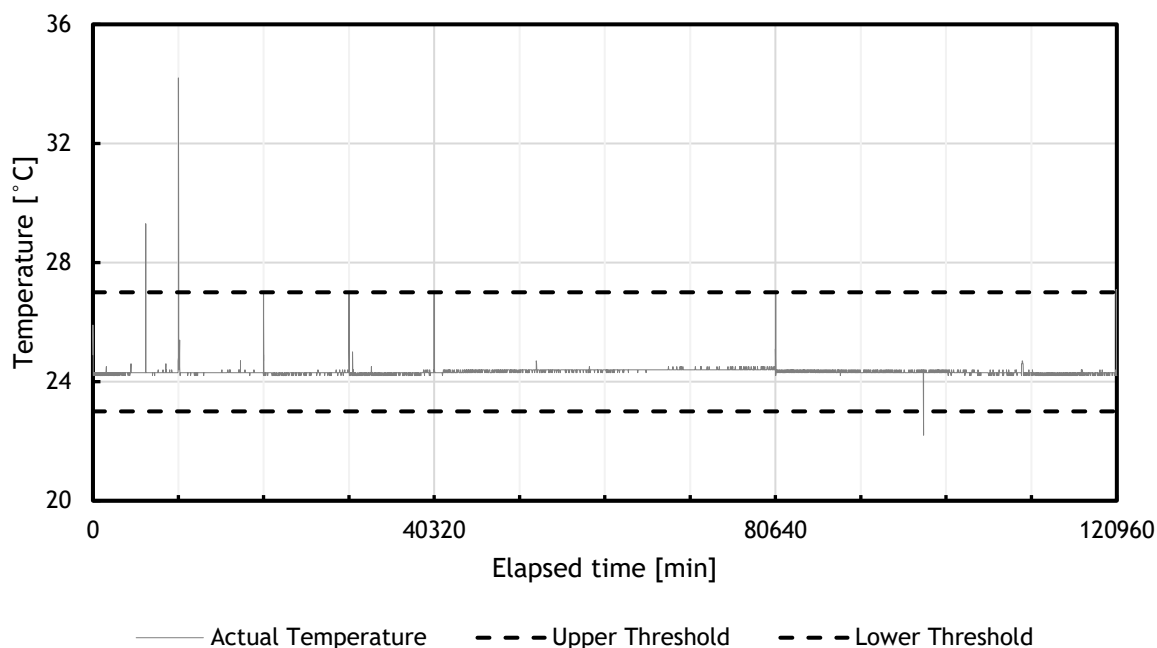


Figure 5—25: Logged temperature for samples stored at 5 °C

Figure 5—25 shows that most of the data points undercutting the lower threshold are at the beginning of the stability study. The reason for this is that the samples infilled a large amount of the space available in the refrigerator. This resulted in the need to adjust the cooling setting of the apparatus several times during the first days of the stability study, as the refrigerator does not cool to a defined temperature but with a certain amount of energy. Less space in the apparatus results in a lower temperature as the same amount of energy is used to cool less air. The upper temperature threshold of the refrigerated storage condition was exceeded for two data points after one week. This happened while the samples were withdrawn for the first time. Overall, 66 data points were out of the range of 2 °C – 8 °C, which corresponds to

about 1.49 % of the recorded data. It must be noted that six scheduled data points were not registered due to a disconnection between the sensor and the logging device.



*Figure 5—26: Logged temperature for samples stored at 25 °C & 60 % rH*

Figure 5—26 shows that the temperature recorded in the climate cabinet of the 25 °C & 60 % rH storage condition was more stable than the temperature logged in the refrigerator. This is due to the operating mode of a climate cabinet. These apparatuses have internal sensors which register temperature and rH. If these sensors register a value which does not conform with the set value, the climate cabinet will countersteer to reach the defined values. Overall, the upper threshold was exceeded six times and the lower threshold undercut once, meaning that circa 0.16 % of the registered data points were out of specification. Five of the six times the upper threshold was exceeded occurred during the withdrawal of samples for content analysis. This is visible as these temperature spikes align with the auxiliary lines. The other two aberrances occurred randomly, possibly due to a malfunction of the internal sensors.

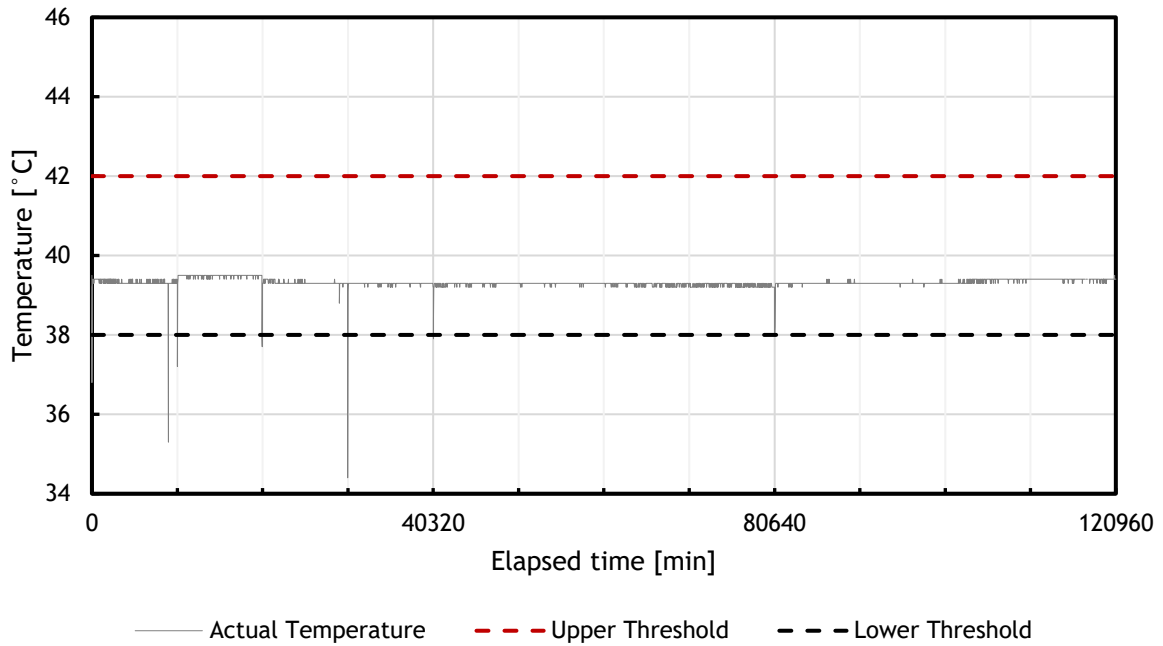


Figure 5—27: Logged temperature for samples stored at 40 °C & 75 % rH

Figure 5—27 depicts the temperature recorded in the climate cabinet set to 40 °C & 75 % rH. Like in the other climate cabinet, the temperature is very stable. The upper limit was not exceeded. The lower threshold, however, was undercut during seven data points, which, again, aligns with the withdrawal of samples for content analysis. This means that about 0.16 % of the recorded data points were out of the specified values. 14 scheduled data points were not registered due to a connection loss between the sensor and the logging device.

## Twelve-Week Stability of the CBD Matrix Particles

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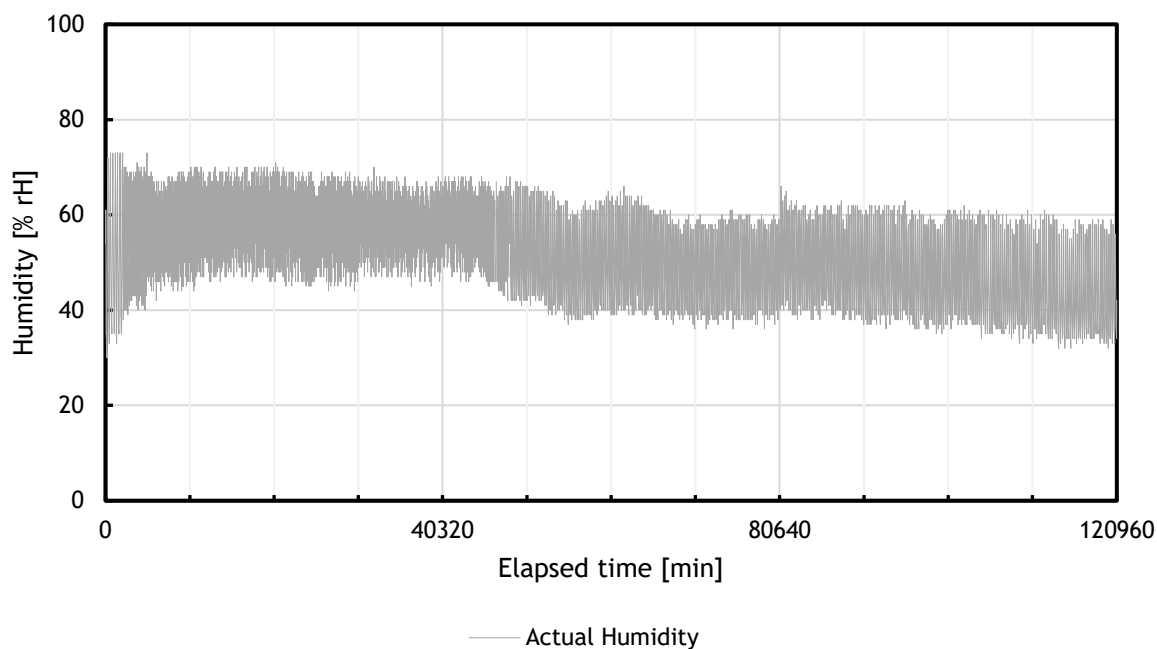


Figure 5—28: Logged rH for samples stored at 5 °C

Figure 5—28 shows the logged rH from the refrigerator. No thresholds are shown in the diagram, as the rH for this storage condition is not specified in the ICH guideline Q1A(R2) [137].

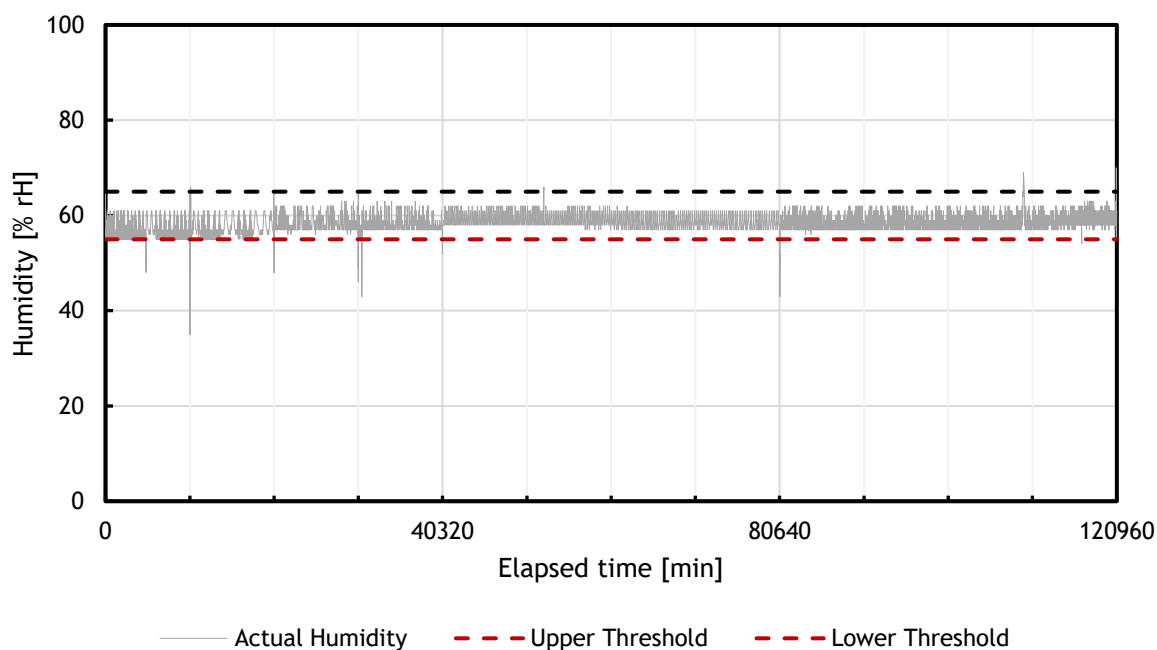


Figure 5—29: Logged rH for samples stored at 25 °C & 60 % rH

Figure 5—29 reveals that, similar to the temperature, the rH achieved in the climate cabinet (here 25 °C & 60 % rH) is more stable than in the refrigerator, the reasons being the same. The upper threshold was exceeded during eight scheduled data points while the lower

threshold was undercut during 23 data points. Like the temperature, most of these aberrances align with the withdrawal of the samples for content analysis. However, the number of points out of specification is higher (circa 0.70 %), as the climate cabinet can adjust the temperature more quickly than the humidity.

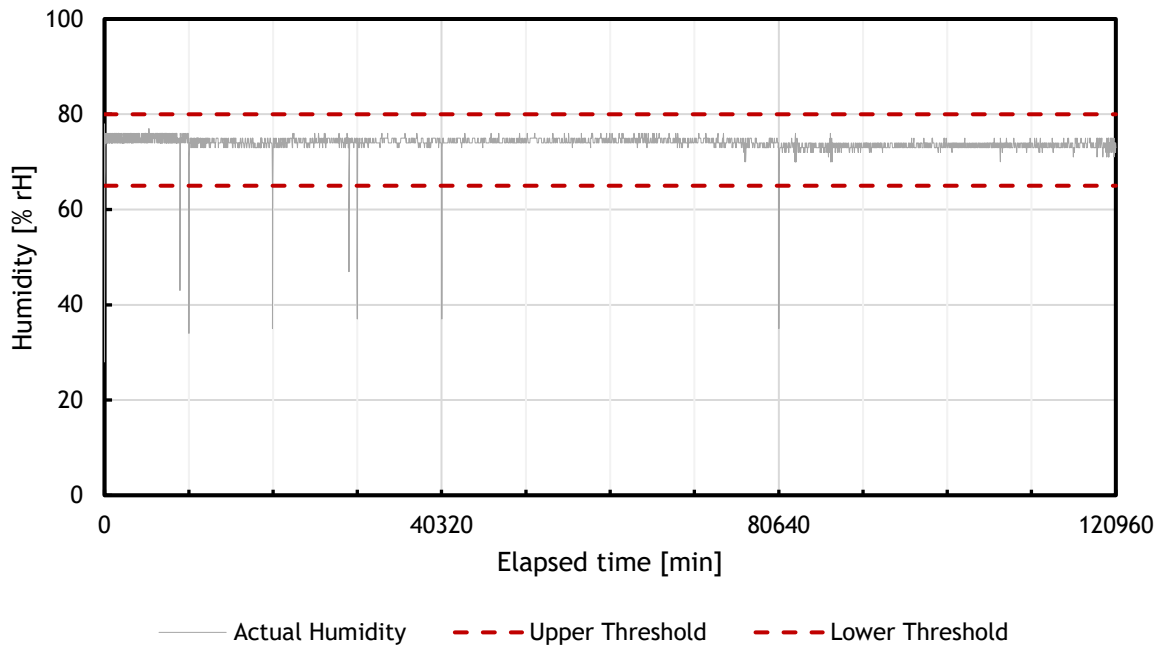


Figure 5—30: Logged rH for samples stored at 40 °C & 75 % rH

Figure 5—30 shows the rH logged in the climate cabinet set to 40 °C & 75 % rH. Again, the aberrances align with the sample withdrawal. However, due to the larger discrepancy between the rH inside apparatus and the exterior, the drops are larger. The upper threshold was not exceeded while 25 data points were below the lower threshold. This means that about 0.57 % of the data points were out of specification. This is acceptable according to the adapted ICH guideline, as short term spikes due to facility opening are accepted as unavoidable and only long term exceedings (> 24 h) have to be reported and individually assessed. [137]

Overall, it can be said that the specifications of the storage conditions were mostly met with only a few data points per recorded condition being out of specification. This can also be seen in Table 5—18. The mean values and the corresponding SD of registered temperature and rH data points are clearly within the thresholds specified in chapter 4.3.2.1.

Table 5—18: Mean values and SD of the logged temperature and rH data

Storage condition	5 °C	25 °C & 60 % rH	40 °C & 75 % rH
Mean temperature ± SD [°C]	4.98 ± 1.51	24.34 ± 0.26	39.33 ± 0.15
Mean rH ± SD [%]	44.9 ± 4.4	58.2 ± 2.04	74.1 ± 2.2

### 5.6.2 CHANGE IN CBD CONTENT DURING TWELVE WEEKS

The following chapters depict the development of the measured CBD contents of the samples during the stability study. The CBD values are depicted as the relative concentration compared to the initial measurement. When multiple concentrations are mentioned in succession, the order is always: 5 °C sample, 25 °C and 60 % rH (25/60) sample, 40 °C and 75 % rH (40/75) sample.

Over the course of the stability study several unforeseeable problems with the analytical equipment occurred. The measurements after two and four weeks were completely excluded from the stability study due to issues with the autosampler, which resulted in the abortion of the analytical sequence at random time points. In addition to these problems, the detector registered incomprehensible signals for the initial calibration curve of the four-week samples.

Furthermore, pressure fluctuations occurred during several analyses, which resulted in a changed elution time of the CBD peak. It can be supposed that this results in the overlapping of the CBD peak and peaks of degradants that elute close to CBD. Therefore, results that had an elution time < 5.8 or > 6.4 were not considered during data evaluation. This resulted in the absence of some measuring points in the diagrams of the following chapters.

Measuring points that are based on less than three values are depicted as bordered white markers. Markers without error bars are based on only one value.

After four weeks the analytical method was re-evaluated, by measuring two different sets of calibration solutions for six consecutive days, to ensure the functionality of the equipment. These experiments confirmed the suitability of the developed method and the replacement autosampler. Additionally, it was shown that concentration differences larger than 1 µg/mL could be detected steadily.

Considering the packaging, it is expected that the blister provides the best protection from the applied conditions, as the formulation is tightly sealed, which inhibits the adhesion of water due to higher applied relative humidities. It is also expected that the capsule provides a certain amount of protection from the environment compared to the bulk storage. However, as the capsule is not as impervious as the aluminium-aluminium blister, changes should be visible. Additionally, it is expected that the addition of AP exerts a positive effect on the degradation of CBD, which means that the degradation velocity should be decreased.

When the different matrices are considered, it is expected that the HPMC-AS matrix is the most resistant to the storage conditions due to its lower hygroscopicity and higher lipophilicity, as this means that less water is available to react with the formulation or the incorporated



CBD. Regarding the stability of the model drug in the different matrices, it is expected that the HPMC matrix is superior in the protection of CBD, as previous experiments of environmental storage suggested a slower degradation velocity in this matrix (compare with 5.3.1 & 5.4.1). As no such experiments were carried out with SP beforehand, an expectation regarding the stability of the API in this matrix cannot be made.

Overall, the evaluation of the stability study is focussed on the values that were measured after 12 weeks and the subsequent UV-irradiation.

### 5.6.2.1 CBD Qualities

Figure 5–31 and Figure 5–32 show the concentration development of the different CBD qualities over the stability study. Both diagrams show values that are fluctuating around 100 %. For the pure CBD samples, every time point yielded at least one sample (storage condition) with an RSD larger than 8.5 %, while the extract always had a sample with an RSD larger than 6.7 %. The relatively large RSDs could be a result of measuring inaccuracies or indicate analytical problems (i.e. pressure fluctuations, see chapter 5.6.2). Mistakes in the sample preparation are also a possibility but improbable, due to a standardised preparation mechanism. Additionally, as the samples are prepared with a highly volatile solvent (acetonitrile), evaporation is a possible explanation.

Pure CBD was used as the standard substance. As the diagrams show the relative concentration of the samples the initial content is set to 100 %. The analysis of the content initial of the CBD extract with this standard yielded an acceptable RSD of < 3 % (2.993). This further substantiates the assumption that further analytical problems are the probable reason for the samples with higher RSDs.

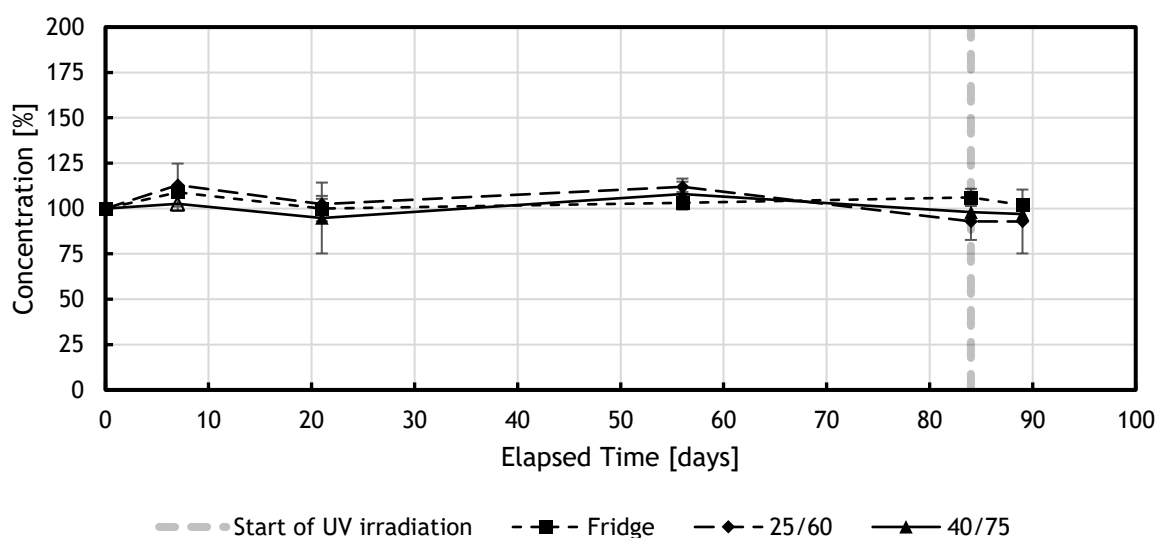


Figure 5–31: Concentration development of pure CBD bulk samples over the stability study;  $n = 3$ ; error bars = RSD

## Twelve-Week Stability of the CBD Matrix Particles

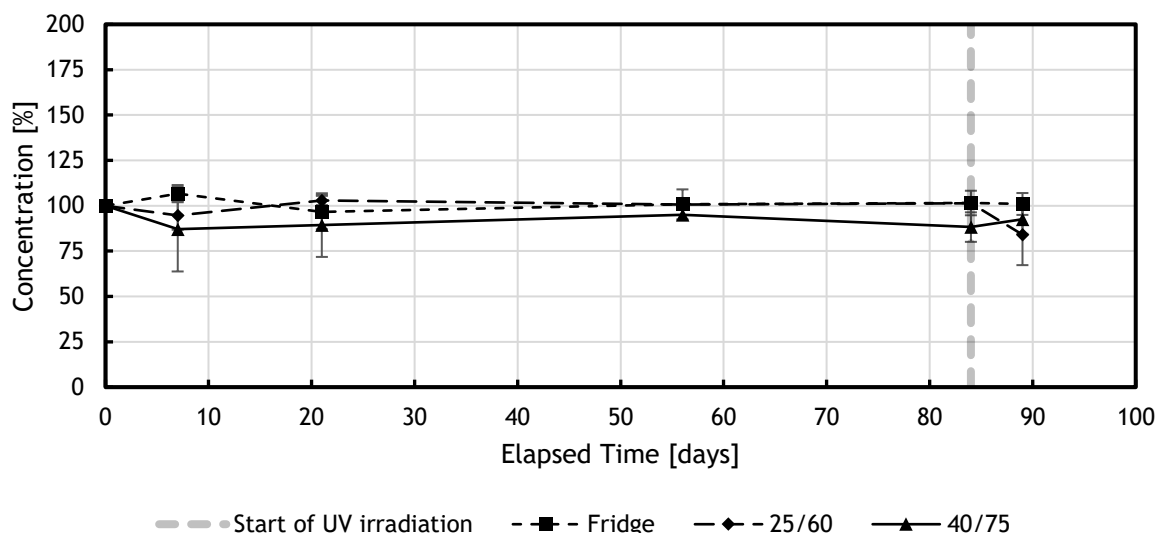


Figure 5—32: Concentration development of CBD extract bulk samples over the stability study;  $n = 3$ ; error bars = RSD

The comparatively constant CBD content of the samples, coupled with relatively high RSDs of some samples, resulted in only three measurements that were significantly different ( $p < 0.031$ ) from the initial value. These were the pure CBD samples that were stored in the refrigerator and taken after one and eight weeks ( $108.9 \pm 1.4\%$  &  $103.1 \pm 1.0\%$ ) and the eight-week sample that was stored at  $25\text{ }^{\circ}\text{C}$  and  $60\%$  rH ( $111.9 \pm 3.2\%$ ). Each of these samples showed a higher CBD content than initially, which could be a result of analytical inaccuracies or solvent evaporation.

The underlying ICH guideline [137] defines a 5% change from the initial value as significant. However, due to the analytical problems and the resulting inaccuracies and therefore high RSDs it can be suspected that crystalline CBD is relatively unsusceptible to the evaluated storage conditions and UV irradiation. After twelve weeks the measured relative concentrations for the pure CBD were  $106.1 \pm 5.0\%$ ,  $92.9 \pm 9.5\%$  and  $98.0 \pm 4.6\%$  ( $p > 0.169$ ) which changed to  $101.9 \pm 2.7\%$ ,  $92.8 \pm 16.4\%$  and  $96.9 \pm 3.8\%$  ( $p > 0.300$ ) after UV-irradiation. The CBD extract showed relative concentrations of  $101.6 \pm 7.3\%$ ,  $101.4 \pm 1.5\%$  and  $88.4 \pm 9.8\%$  ( $p > 0.058$ ) after twelve weeks and  $100.9 \pm 3.1\%$ ,  $83.9 \pm 12.1\%$  and  $92.8 \pm 10.1\%$  after UV irradiation ( $p > 0.066$ ).

Figure 5—33 and Figure 5—34 show the concentration progression of the solutions prepared from the CBD qualities. The measured relative concentrations fluctuate around 100%. However, some measurements stray relatively far from 100% with high RSDs, whereas the following measurement shows a higher concentration. This, again, signals analytical inaccuracies, probably due to shifting retention times.

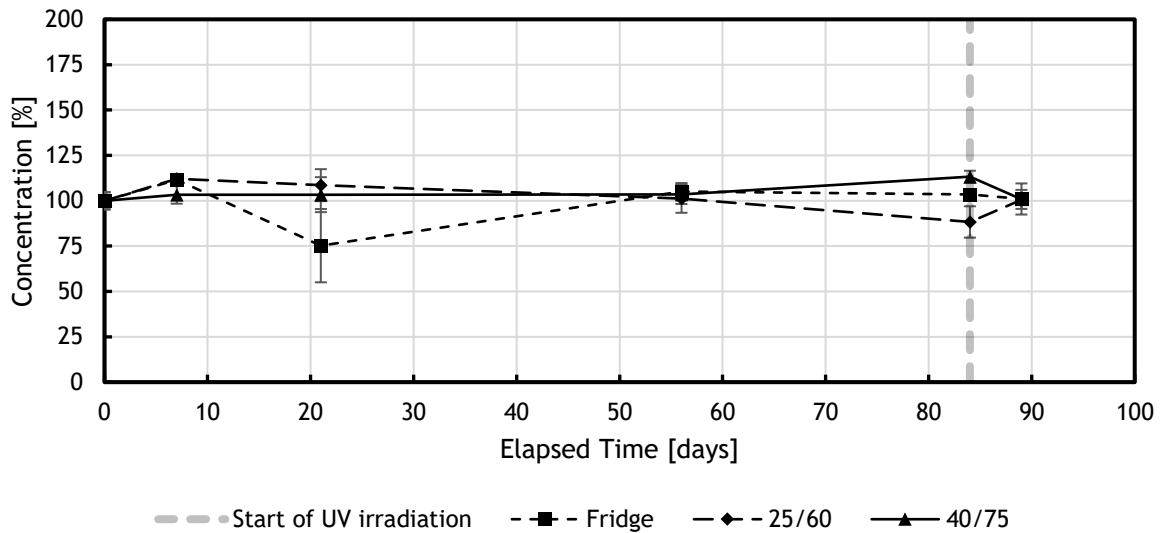


Figure 5–33: Concentration development of the pure CBD solutions over the stability study;  $n = 3$ ; error bars = RSD

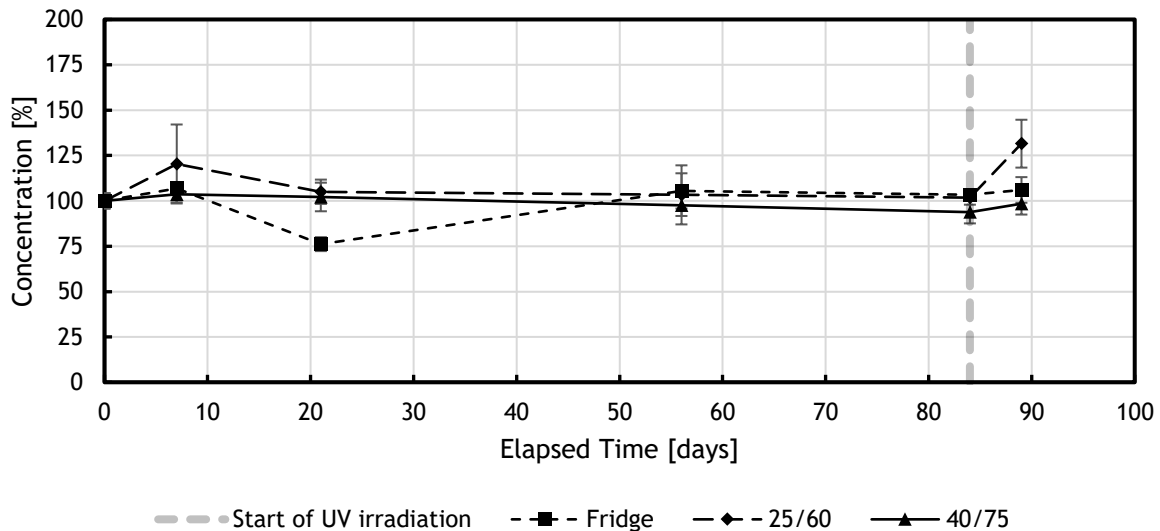


Figure 5–34: Concentration development of the CBD extract solutions over the stability study;  $n = 3$ ; error bars = RSD

After twelve weeks, the pure CBD solution showed relative concentrations of  $103.5 \pm 3.1\%$ ,  $88.2 \pm 3.8\%$  and  $113.4 \pm 2.9\%$ . The 40/75 samples' aberration was significant ( $p = 0.019$ ). After the subsequent UV irradiation, the concentrations were  $101.0 \pm 8.6\%$ ,  $100.9 \pm 4.0\%$  and  $100.8 \pm 3.7\%$  ( $p > 0.860$ ). For the solution of the CBD extract, the found concentrations were  $103.5 \pm 2.3\%$ ,  $101.9 \pm 0.4\%$  and  $93.7 \pm 2.0\%$  ( $p > 0.206$ ) after twelve weeks and  $105.9 \pm 3.2\%$ ,  $131.8 \pm 19.2\%$  and  $98.5 \pm 3.9\%$  after UV irradiation. The 25/60 sample was significantly ( $p = 0.038$ ) higher than the initial measurement.

The samples with significantly higher CBD contents indicate measurement inaccuracies or solvent evaporation, as it is highly improbable that new CBD was formed during UV irradiation.

Although the difference between some of the values are > 5 %, it can be assumed that CBD solutions are relatively stable, when stored in darkness, as no value showed a significantly lower relative concentration. However, it was expected that the subsequent UV irradiation would lead to a loss of CBD. Two possible explanations for the missing reduction are the evaporation of solvent and that the SCGs (soda-lime glass) are mostly non-transparent for UV light [145].

### 5.6.2.2 pMDIs

Figure 5–35 and Figure 5–36 show the measured relative CBD concentrations of the two pMDI formulations over the course of the twelve-week stability study. It is striking that the measured concentrations show a seemingly random development. Again, Some of the datapoints show very large standard deviations.

Due to a lack of space in the UV chamber and the complete imperviousness of the aluminium cans to light, the pMDIs were excluded from the subsequent UV-irradiation. This results in one datapoint less than found in the other diagrams.

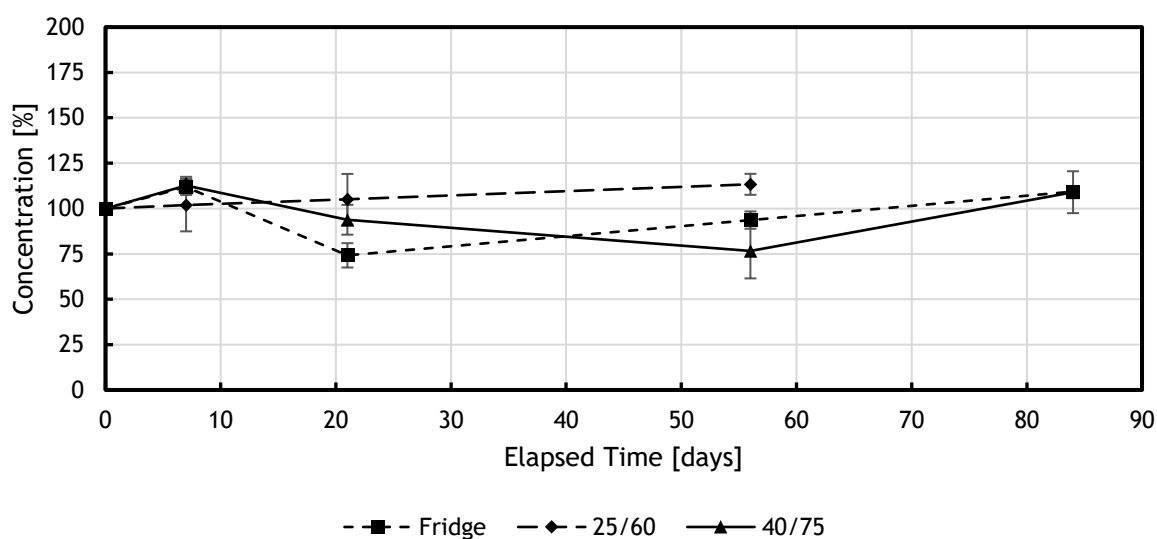


Figure 5–35: Concentration development of the CBD pMDIs over the stability study;  $n = 3$ ; error bars = RSD

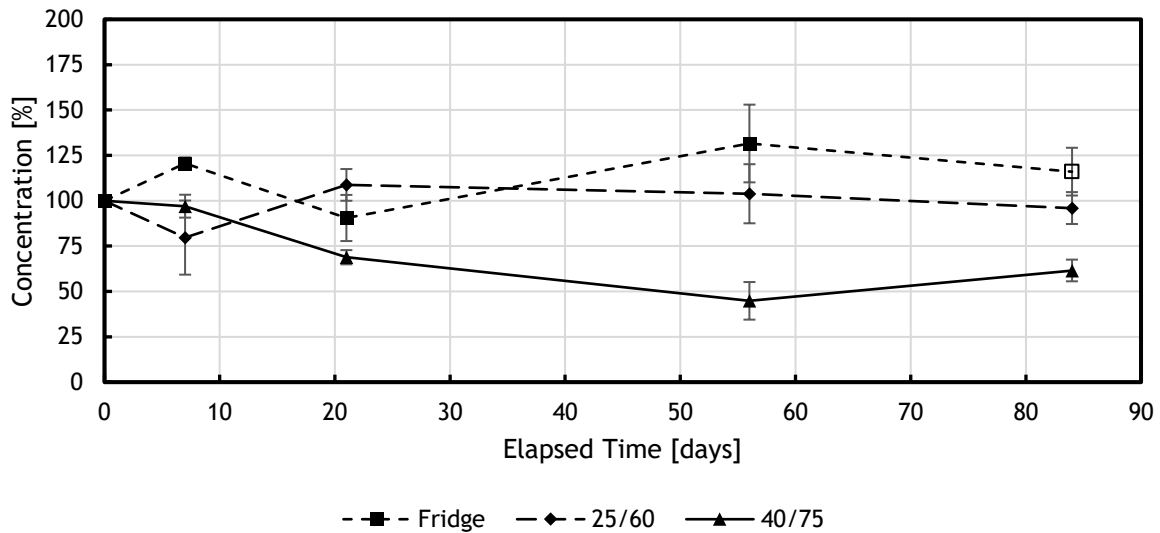


Figure 5–36: Concentration development of the CBD + AP pMDIs over the stability study;  $n = 3$ ; error bars = RSD

After twelve weeks, the basic pMDI was evaluated to have relative CBD concentrations of  $109.4 \pm 2.4 \%$  ( $p = 0.018$ ),  $113.3 \pm 6.6 \%$  (after 8 weeks;  $p = 0.028$ ) and  $109.0 \pm 12.3 \%$  (40/75;  $p = 0.341$ ). The 12 week 25/60 samples had to be excluded due to the aforementioned pressure fluctuations during analytics. Due to the fact that every sample had a measured concentration of  $> 100 \%$  it can be assumed that CBD is relatively stable in this formulation. However, the increased CBD contents could also be a result of malfunctioning dosing valves or an unsuited preparation method.

The pMDI with AP, stored at  $40 \text{ }^\circ\text{C}$  and  $75 \%$  rH showed distinctly lower concentrations from week three on. This could be a result of the fact that the AP was not completely soluble in this formulation. This was observable, as the preparation of the samples after actuation yielded suspensions. The undissolved AP could have blocked the dosing chamber, which would result in a smaller volume of the formulation being emitted from the can. If the expelled volume of formulation is unknowingly lower, the standardised dissolution steps result in lower concentrations of the samples. These problems could maybe have been avoided by using an alternative AO for the pMDI formulation. Due to the relatively low lipophilicity of HFA 134a ( $\log P$  2.27 [146]) compared to AP ( $\log P$  8.21 [107]), the use of AA ( $\log P$  -0.18 [104]) could have yielded better results, while maintaining the same type of AO.

After twelve weeks the found concentrations were  $115.3 \pm 16.2 \%$ ,  $96.0 \pm 8.9 \%$  and  $61.6 \pm 4.1 \%$ . The 40/75 sample ( $p < 0.001$ ) has to be evaluated critically, for aforementioned reasons. The higher value of the refrigerated sample could, as with the basic formulation, be a result of an unsuitable preparation method, retention time shifts (peak overlapping) or a malfunctioning

dosing valve. Additionally, it was found out that AP is not suitable for HFA 134a formulations, as the needed amounts are not soluble in this propellant/solvent combination.

### 5.6.2.3 HPMC Formulations

As seen with the pure CBD measurements, some measurements show large RSDs (up to 29.0 %). Additionally, some samples showed (significantly) higher concentrations than initially. However, these were primarily seen with the blister samples. Here, it has to be kept in mind, that it was not possible to use an initial weight for the calculation of the remaining CBD content, as the blister cups were filled volumetrically. Therefore, it is possible that an increased CBD content was measured due to changes in the formulation (e.g. lower moisture content due to sealing process).

As it is expected that the degradation of CBD follows an oxidative route, the reduction rate of the API should be constant [103]. However, the build-up of the solid dispersions could result in a decelerating degradation velocity, as the CBD that is located near the centre of the individual particles could be less accessible for the oxygen or other reacting molecules (see Figure 5—2 to Figure 5—4). Additionally, it can be suspected that storage in the refrigerator leads to lower degradation rates, as oxidative reactions are known to proceed slower at lower temperatures [103]. These expectations were not entirely met by the HPMC formulations. The only samples that were similar to the expectation were the 40/75 bulk and capsule samples of the basic formulation, as the CBD content decreased more rapidly during the first two weeks before reaching a relatively constant rate.

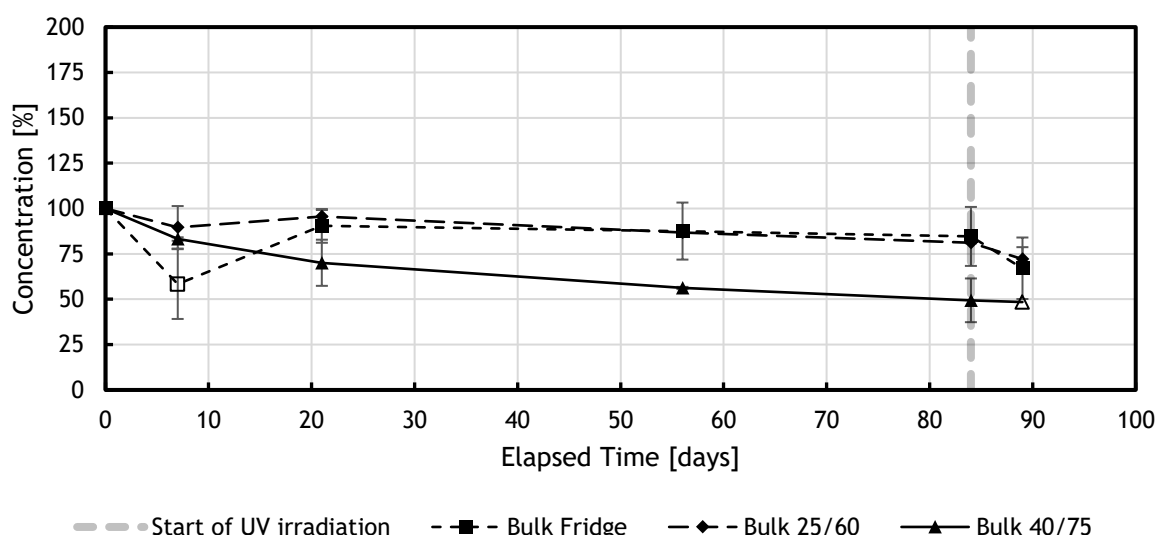


Figure 5—37: Concentration development of the HPMC + CBD bulk samples over the stability study;  $n = 3$ ; error bars = RSD

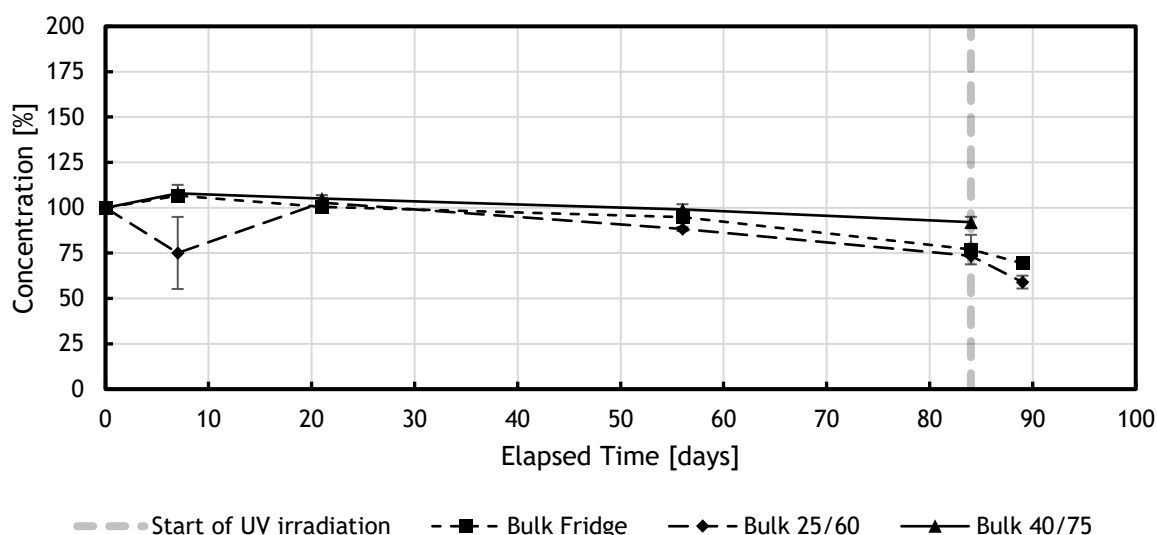


Figure 5—38: Concentration development of the HPMC + CBD + AP bulk samples over the stability study;  $n = 3$ ; error bars = RSD

Figure 5—37 to Figure 5—42 show the concentration development of both HPMC formulations over the twelve week stability study with subsequent 5 days of UV-irradiation for each defined storage condition.

The bulk samples (Figure 5—37 & 38) generally showed a somewhat random development, as some measurements have a higher mean concentration as the previous value. Especially the results generated after one week were lower and exhibit a higher RSD. As stated in chapter 5.6.2 there were several unforeseeable analytical problems during this study. Therefore, these measurements have to be evaluated carefully, especially as the progression after week one was comparatively linear. After twelve weeks, the basic formulation showed relative concentrations of  $84.6 \pm 13.7\%$ ,  $81.1 \pm 0.3\%$  and  $49.4 \pm 5.9\%$ , while the formulation with AP had concentrations of  $76.9 \pm 6.3\%$ ,  $73.6 \pm 0.5\%$  and  $92.1 \pm 2.7\%$ . Except for the refrigerated sample of the basic formulation, each sample showed a significantly ( $p < 0.024$ ) lower concentration after twelve weeks than initially.

When the post-stability concentrations are compared among each other, it is visible, that the degradation of the basic formulation was, as expected, accelerated at more stressful conditions, as both the refrigerated and the 25/60 post-stability samples showed a higher CBD content than the 40/75 sample ( $p < 0.016$ ). Interestingly, this seems to be reversed when AP is added to the formulation, as the 40/75 samples of this formulation showed a higher ( $p < 0.019$ ) relative post-stability concentration than the samples of both other storage conditions. This was unexpected, as more stressful conditions should result in a higher degradation velocity. However, this was only observed for the HPMC formulations (see chapters 5.6.2.4 & 5.6.2.5).

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This is confirmed by comparing the different formulations at the same storage condition. The mean content values for the basic formulation showed higher end concentrations after twelve weeks, when stored in the refrigerator ( $p = 0.447$ ) or at 25 °C and 60 % rH ( $p < 0.001$ ). After storage at 40 °C and 75 % rH the AP formulation had a significantly higher content ( $p < 0.001$ ).

Subsequent UV irradiation resulted in concentrations of  $67.1 \pm 11.4$  %,  $72.1 \pm 4.7$  % and  $48.5$  % ( $n = 1$ ) for the basic formulation and  $69.4 \pm 0.8$  % and  $59.0 \pm 2.1$  % (the 40/75 samples were excluded) for the formulation with the A0. The 25/60 sample of the AP formulation had a lower ( $p < 0.001$ ) concentration after UV compared to the post-stability measurement.

Nonetheless, comparison of the formulations revealed that the 25/60 base sample had a higher ( $p = 0.012$ ) concentration. The refrigerated samples showed no difference ( $p = 0.744$ ), whereas a comparison of the 40/75 samples was not possible as only one measurement of the basic formulation could be included.

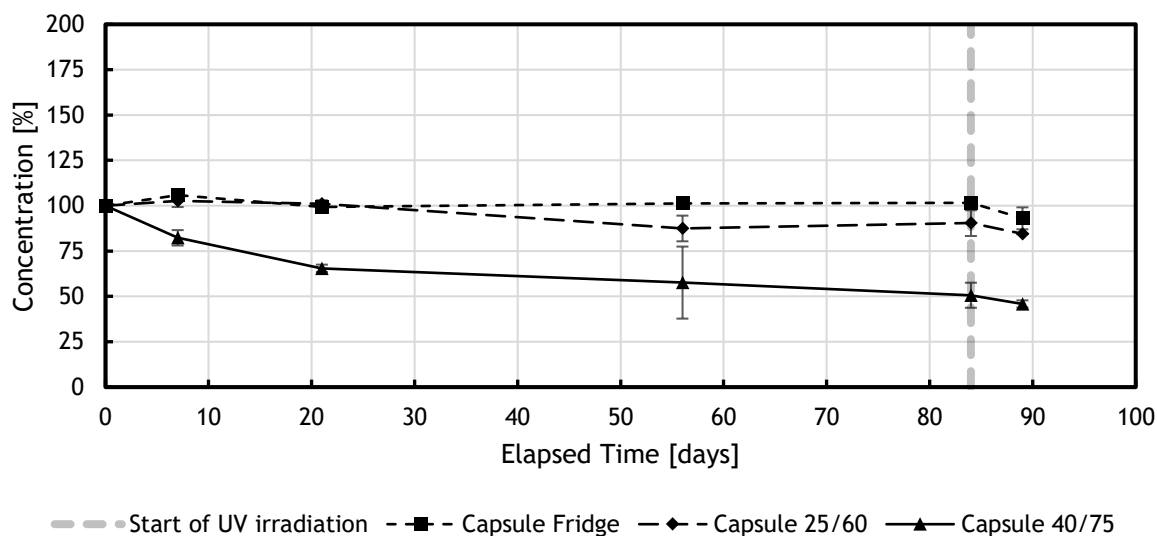


Figure 5–39: Concentration development of the HPMC + CBD capsule samples over the stability study;  $n = 3$ ; error bars = RSD



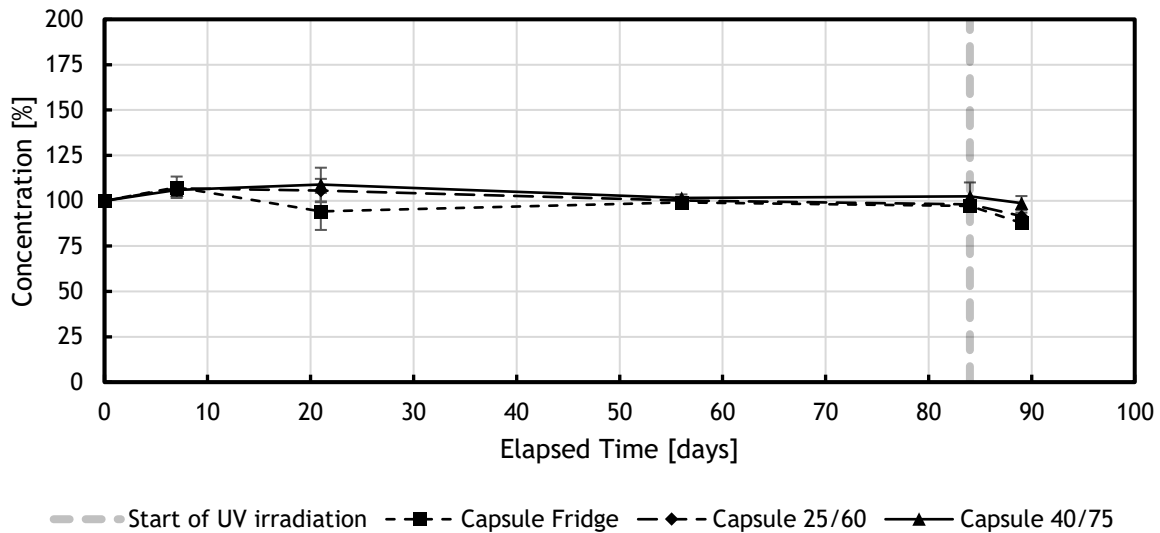


Figure 5—40: Concentration development of the HPMC + CBD + AP capsule samples over the stability study;  $n = 3$ ; error bars = RSD

The capsule samples (Figure 5—39 & 40), like the bulk samples, followed a somewhat random progression, as some measured values were higher than the previous measurement. However, it can be assumed, that the capsule has protective properties on the AP formulation, as all measurements are relatively stable at about 100 %.

After twelve weeks the basic formulation had concentrations of  $101.7 \pm 0.7 \%$ ,  $90.4 \pm 6.5 \%$  and  $50.7 \pm 3.6 \%$ . The corresponding values for the formulation with AP were  $97.1 \pm 2.4 \%$ ,  $98.0 \pm 1.2 \%$  and  $102.5 \pm 7.8 \%$ . Every capsule sample of the formulation with AP and the 25/60 sample of the basic formulation was not different from the initial measurement ( $p > 0.099$ ), while the refrigerated basic sample showed a higher concentration ( $p = 0.008$ ) than initially, whereas the 40/75 sample had a significantly lower ( $p = 0.002$ ) content.

Post-stability, differences were observable between the 40/75 samples of the basic formulation and the samples of both of the other storage types ( $p < 0.002$ ). No differences were visual between the capsule samples of the AP formulation at the different storage conditions. This shows that the combination of AP and the capsule exhibit an effective protection. As expected, the degradation was faster at more stressful conditions for the basic formulation.

Comparing the formulations at the same storage condition show no differences ( $p = 0.118$ ), when stored at 25 °C and 60 % rH, while the AP formulation had a lower content after storage in the refrigerator ( $p = 0.032$ ) and a higher remaining concentration after storage at 40 °C and 75 % rH ( $p = 0.001$ ),

After UV irradiation, the relative CBD contents were evaluated to be  $93.1 \pm 5.7 \%$ ,  $84.5 \pm 0.3 \%$  and  $45.9 \pm 1.0 \%$  for the basic formulation and  $87.8 \pm 2.9 \%$ ,  $91.6 \pm 1.7 \%$  and  $98.7 \pm 3.9 \%$  for the

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formulation with AP. The refrigerated base sample and the 40/75 sample of the AO formulation showed no difference ( $p > 0.166$ ) to the initial measurement, while every other sample showed significantly ( $p < 0.019$ ) lower relative CBD contents. UV-irradiation did not yield any different ( $p > 0.087$ ) concentrations to the post-stability measurement for the basic formulation. The formulation with AP had reduced ( $p < 0.013$ ) CBD contents for the refrigerated and 25/60 samples.

Interestingly, the comparison of the formulations after UV irradiation shows no difference after previous storage in the refrigerator ( $p = 0.226$ ), while the 25/60 and 40/75 samples were higher for the AP formulation ( $p < 0.018$ ). Before the UV-irradiation the refrigerated and 40/75 samples were significantly different. This difference could be a result of the analytical inaccuracies or a result of an uneven UV-irradiation.

Comparison of the relative concentrations of the basic formulations bulk and capsule samples after twelve weeks showed no differences ( $p > 0.130$ ) at any storage condition. When the same statistical evaluation was applied to the samples of the formulation with AP, it could be observed that the capsule samples stored in the refrigerator or at 25 °C and 60 % rH had a higher ( $p < 0.006$ ) remaining concentration than the corresponding bulk samples.

The same comparison of the post-UV concentrations shows that the capsules had higher ( $p < 0.044$ ) concentrations for the refrigerated and 25/60 samples of both formulations.

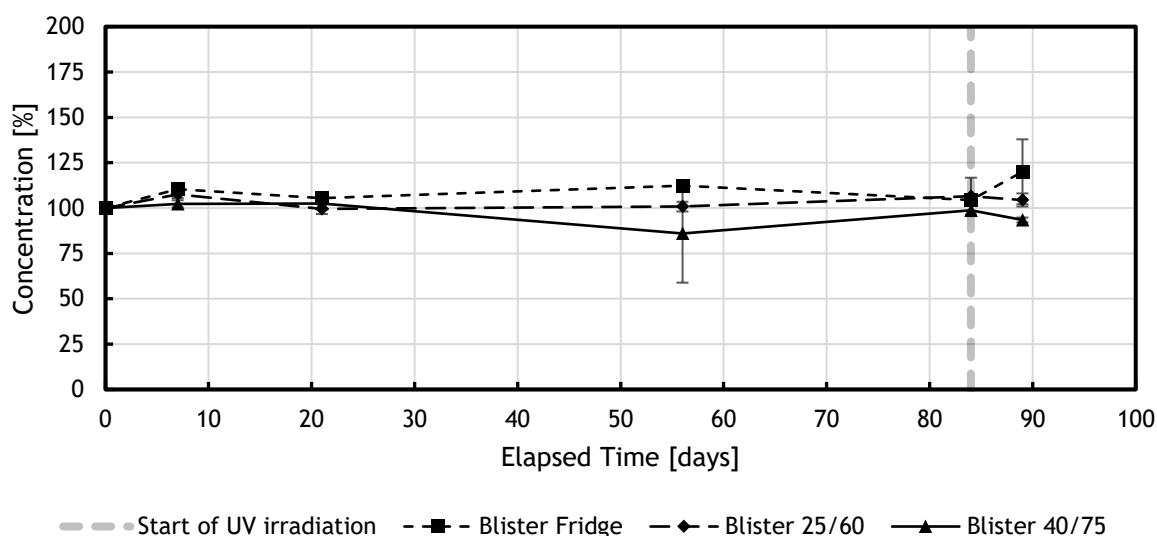


Figure 5—41: Concentration development of the HPMC + CBD blister samples over the stability study;  $n = 3$ ; error bars = RSD

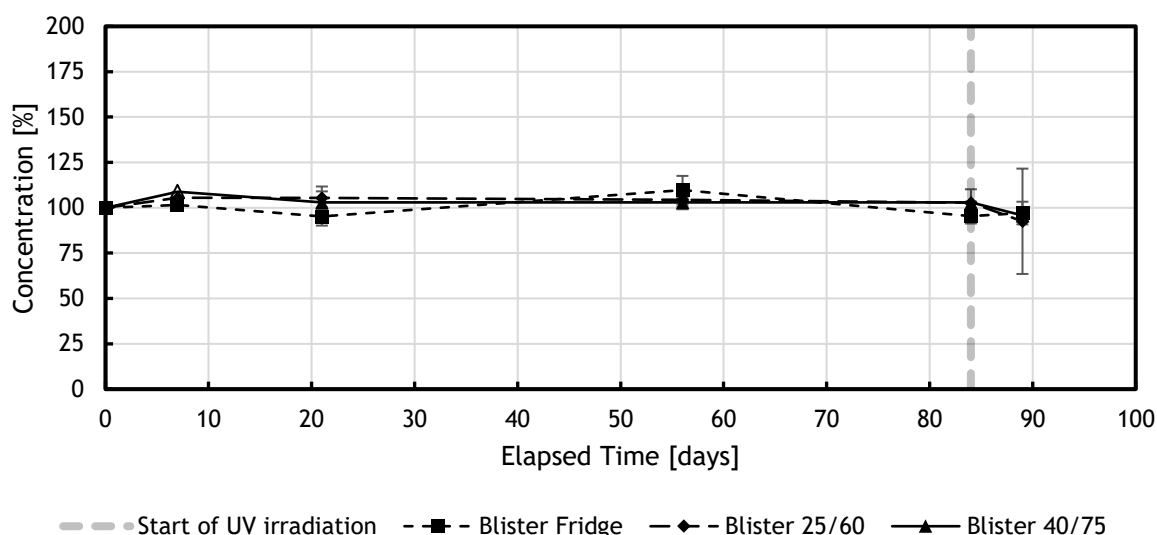


Figure 5–42: Concentration development of the HPMC + CBD + AP blister samples over the stability study;  $n = 3$ ; error bars = RSD

The blister samples (Figure 5–41 & 42) of both formulations fluctuate around a relative concentration of 100 %. Several of the measurements yielded concentrations above 100 % this could be due to measuring inaccuracies. However, it was visible that the packaging into the blister influenced the formulation. Therefore, it could also be possible that the 180 °C that were applied during the sealing of the blisters reduced the amount of remaining solvents (water/EtOH). This would increase the content in the formulation, resulting in higher measured concentrations. As the actual filling weight of the individual cups is unknown and each sample had to be weighed before content analysis, this assumption is only valid for the blister samples.

The post-stability samples of the basic formulation showed remaining relative concentrations of  $104.3 \pm 0.6 \%$ ,  $106.6 \pm 10.9 \%$  and  $98.7 \pm 3.1 \%$ . The corresponding values for the AO formulation were  $95.4 \pm 3.8 \%$ ,  $102.8 \pm 0.6 \%$  and  $102.9 \pm 7.6 \%$ . The only significant ( $p < 0.018$ ) aberrations to the initial measurement could be observed for the refrigerated samples of the basic formulation as well as the 25/60 samples of the AP formulation.

The comparison of the different storage conditions showed significant differences ( $p > 0.039$ ) between the refrigerated and 40/75 samples of the basic formulation as well as between the refrigerated and 25/60 samples of the AP formulation. There were no discrepancies between the formulations at the same storage conditions ( $p > 0.052$ ). This indicates that the blister is providing excellent protection for both formulations and that the AO has no negative effect on the CBD when the formulation is protected from the environment.

After UV irradiation of the blisters, the basic formulation showed concentrations of  $120.0 \pm 21.5 \%$ ,  $104.4 \pm 3.7 \%$  and  $93.4 \pm 1.4 \%$ . The values of the formulation with AP were  $97.1 \pm 6.0 \%$ ,  $92.5 \pm 26.9 \%$  and  $95.8 \pm 1.7 \%$ . Of these measurements, only the 40/75 samples were different ( $p < 0.049$ ) from the initial value. When the post-UV samples are compared to the corresponding post-stability samples, it could be seen that there were no significant ( $p > 0.055$ ) differences at any storage condition, which was expected, as the blister is impervious to light.

As with the post-stability samples, no difference ( $p > 0.052$ ) could be seen between the formulations.

The comparison of the post-stability bulk and blister samples showed differences of the 40/75 samples of the basic formulation (blister more stable;  $p < 0.001$ ), while the refrigerated and 25/60 samples of the A0 formulation showed significant discrepancies (blister more stable;  $p < 0.121$ ). When the blister samples were compared with the corresponding capsule samples, differences were observable between the refrigerated and 40/75 samples of the basic formulation as well as the 25/60 samples of the formulation with AP (blister more stable;  $p < 0.007$ ). The same evaluation of the post-UV samples yielded differences between the refrigerated and 25/60 bulk and blister samples of the basic formulation (blister more stable; not possible for 40/75, as only one post-UV could be included) as well the refrigerated (blister more stable) samples of the formulation with AP ( $p < 0.015$ ). There were no differences between capsule and blister of the AP formulation. However, the 25/60 and 40/75 samples of the basic formulation showed significant discrepancies (blister more stable;  $p < 0.011$ ).

Ultimately, data for these formulations suggest that the AP is not necessary to protect the CBD in solid dispersions with HPMC as the matrix when the formulation is stored under refrigeration. The optimal packaging for these formulations was the blister, as expected. However, as it is possible that the sealing of the blister had a negative effect on the formulation, it would be better if the formulation would be packaged into a capsule and subsequently in a blister. As the addition of AP did not yield benefits at every storage condition, it can be concluded that the basic formulation is superior if stored and packaged accordingly.

### *5.6.2.4 HPMC-AS Formulations*

As seen before, several samples had very high RSDs (up to 42.1 %), especially after one week. Most of the samples follow a comparatively linear progression, which was expected due to the nature of chemical degradations.

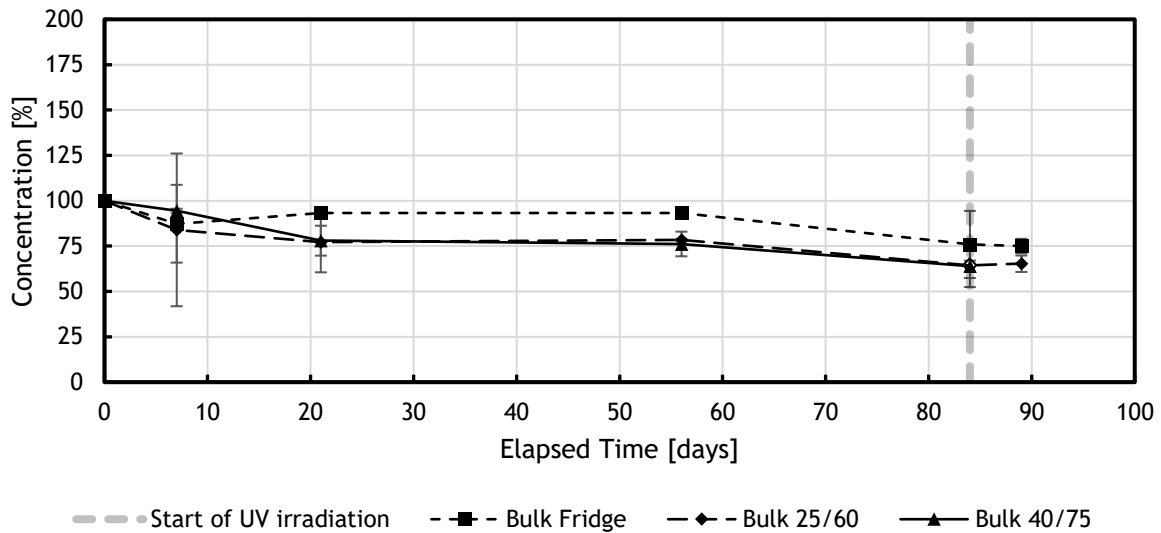


Figure 5—43: Concentration development of the HPMC-AS + CBD bulk samples over the stability study;  $n = 3$ ; error bars = RSD

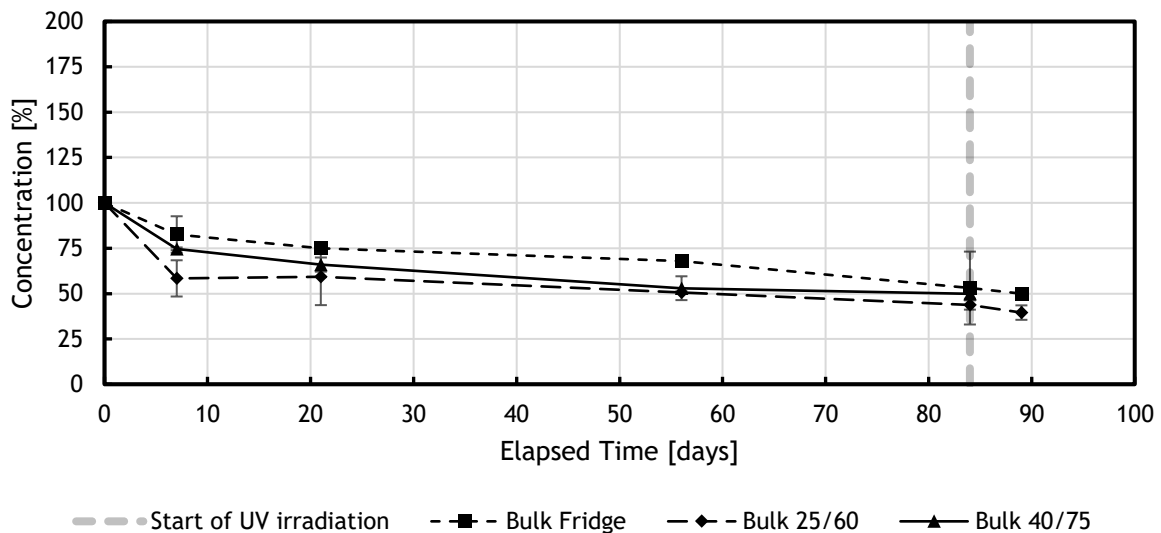


Figure 5—44: Concentration development of the HPMC-AS + CBD + AP bulk samples over the stability study;  $n = 3$ ; error bars = RSD

Figure 5—43 & 44 show the development of the bulk samples of both formulations. After twelve weeks, the samples of the basic formulation had remaining relative CBD contents of  $75.9 \pm 14.0\%$ ,  $64.4 \pm 7.7\%$  ( $n = 2$ ) and  $64.0 \pm 1.9\%$ . Only the 40/75 sample was different from the initial measurement ( $p = 0.001$ ). The formulation with AP had post-stability concentrations of  $53.1 \pm 10.7\%$ ,  $43.7 \pm 1.1\%$  and  $49.9 \pm 1.4\%$ . Each of these measurements was significantly lower ( $p < 0.017$ ) than the initial CBD concentration.

Comparing the relative post-stability concentrations of the bulk samples at different storage conditions yields no differences for basic formulation ( $p > 0.277$ ). However, the 25/60 and 40/75 samples of the formulation with the AO had different ( $p = 0.004$ ) concentrations after

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twelve weeks. Interestingly, the 40/75 sample had the higher concentration of these two samples

The comparison of both formulations bulk samples stored under the same conditions reveals that the addition of AP enhanced the degradation of CBD at more stressful conditions. There were no differences between the refrigerated or 25/60 samples ( $p > 0.093$ ), whereas the difference between the 40/75 samples was significant ( $p < 0.001$ ).

The post-UV bulk samples of the basic formulation had contents of  $75.0 \pm 3.0 \%$  and  $65.3 \pm \%$  (the 40/75 samples were excluded), whereas the formulation with AP showed much lower values of  $50.0 \pm 1.2$  and  $39.5 \pm 1.6 \%$  (the 40/75 samples were excluded). Compared to the initial contents each sample of both formulations had a significantly decreased CBD content ( $p < 0.005$ )

The application of UV light to the post-stability samples had a significant ( $p < 0.020$ ) influence on the 25/60 sample of the formulation with the A0.

Statistical evaluation of the post-UV bulk samples shows that the remaining CBD concentration of the formulation with AP were lower than the corresponding values of the basic formulation ( $p < 0.001$ ).

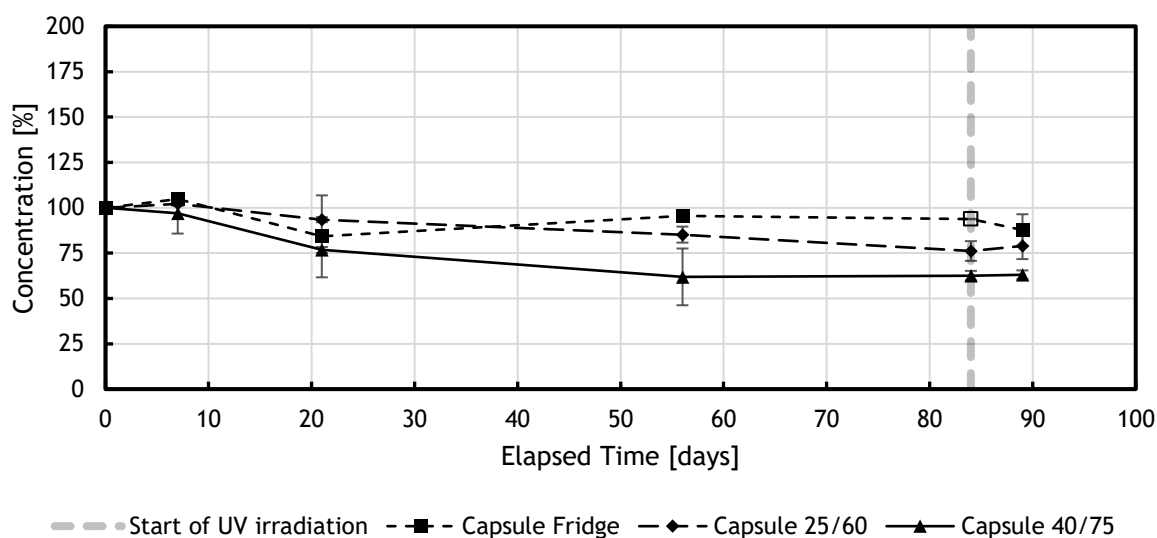


Figure 5–45: Concentration development of the HPMC-AS + CBD capsule samples over the stability study;  $n = 3$ ; error bars = RSD

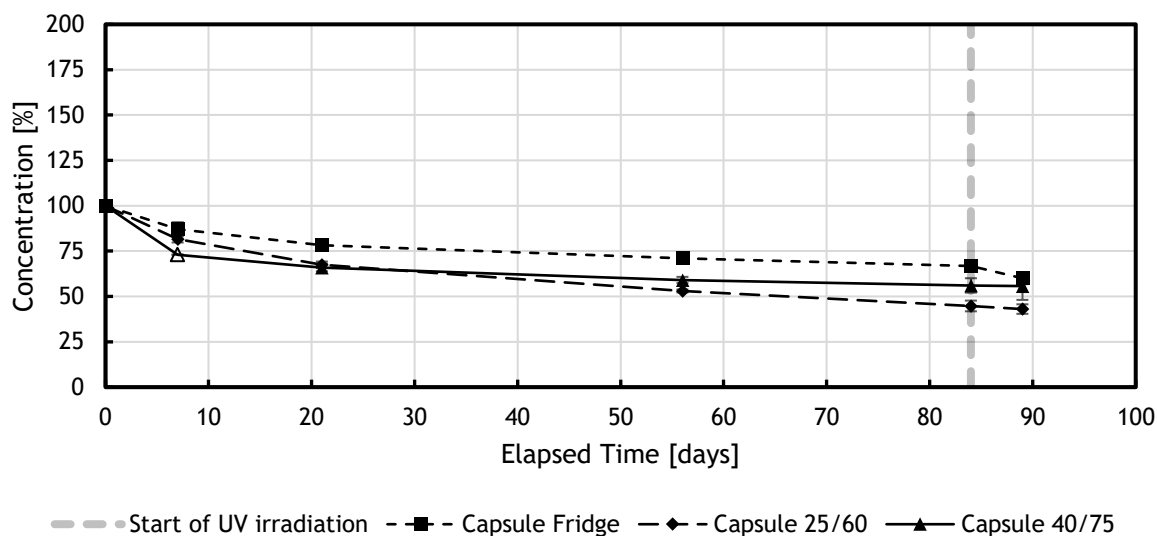


Figure 5—46: Concentration development of the HPMC-AS + CBD + AP capsule samples over the stability study;  $n = 3$ ; error bars = RSD

Figure 5—45 & 46 show the development of the capsule samples. The samples of the formulation with AP followed the expected progression. However, the 40/75 sample of the same formulation showed a slower degradation than the 25/60 sample, which was unexpected. The capsule samples of the HPMC-AS formulation with CBD mostly showed relatively small RSDs, which results in the most significantly different values compared to the respective initial concentration.

The post-stability concentrations that were identified for the basic formulation were  $93.7 \pm 0.1 \%$  ( $n = 2$ ),  $76.1 \pm 4.1 \%$  and  $62.6 \pm 1.6 \%$ . The corresponding values for the AO formulation were  $66.8 \pm 1.6 \%$ ,  $44.8 \pm 1.3 \%$  and  $56.1 \pm 2.2 \%$ . Every value, with the exception of the refrigerated basic sample, was significantly lower than the initial measurement ( $p < 0.010$ ).

When the capsule samples at different storage conditions were compared with each other, differences could be observed for each pairing ( $p > 0.011$ ). However, it has to be remembered, that the remaining content of the 25/60 sample of the formulation with AP is lower than the 40/75 sample. This was also seen with the bulk samples, which unexpectedly indicates that the protective effect of the AP is higher at more stressful conditions.

Comparing the twelve-week samples of both formulations with each other revealed that the addition of AP is counterproductive for the stability of CBD in a HPMC-AS matrix, as every concentration of the basic formulation was higher ( $p < 0.015$ ).

After UV irradiation, the basic formulation showed concentrations of  $87.7 \pm 7.6 \%$ ,  $78.9 \pm 5.7 \%$  and  $63.1 \pm 1.5 \%$ . For the AO formulation the subsequent UV irradiation resulted in relative

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concentrations of  $60.0 \pm 1.9\%$ ,  $43.1 \pm 1.1\%$  and  $55.7 \pm 4.2\%$ . In contrast to the initial measurement, each value was lower ( $p < 0.024$ ), except for the refrigerated sample of the basic formulations ( $p = 0.108$ ). When the post UV samples are compared with the twelve-week samples, the only difference ( $p = 0.001$ ) could be seen between the refrigerated samples of the AP formulation.

When the post-UV capsule samples of the different formulations are compared with each other, it is revealed, that the basic formulation had significantly ( $p < 0.048$ ) higher remaining CBD concentrations, than the formulation with AP, regardless of the storage condition.

The comparison of the bulk and capsule samples of the same storage conditions showed no differences ( $p > 0.104$ ) for any pairing of both formulations, except for the 40/75 samples of the AO formulation. Here the capsule showed a protective effect, as the capsule sample had a higher ( $p = 0.014$ ) relative CBD concentration than the bulk sample. After UV irradiation differences ( $p < 0.021$ ) could be observed for the 25/60 (capsule higher) pairing of the basic formulation as well as the refrigerated and 25/60 samples (capsule higher) of the second formulation ( $p < 0.036$ ).

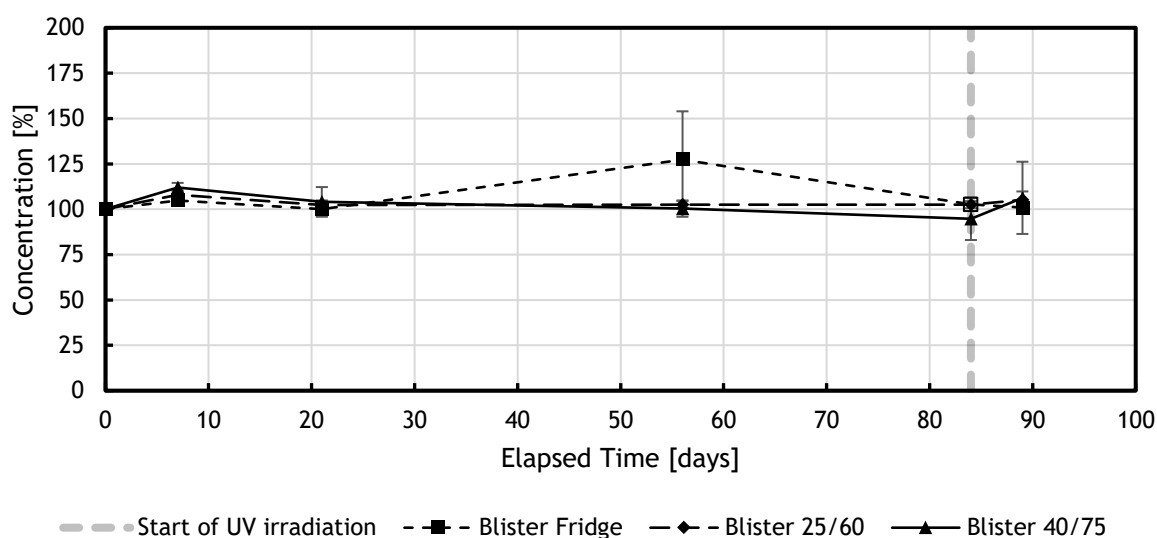


Figure 5—47: Concentration development of the HPMC-AS + CBD blister samples over the stability study;  $n = 3$ ; error bars = RSD



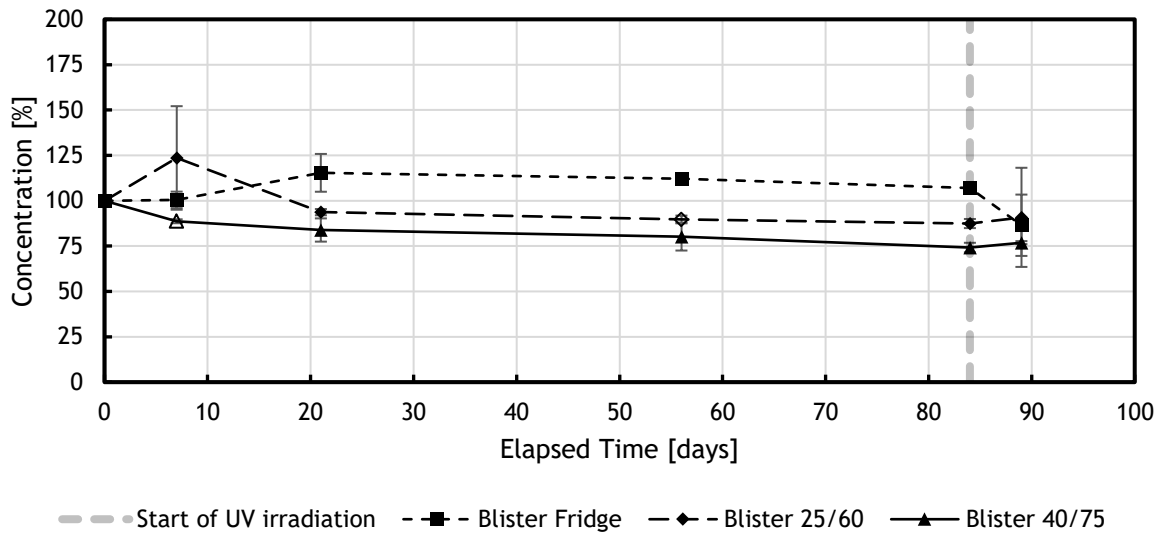


Figure 5–48: Concentration development of the HPMC-AS + CBD + AP blister samples over the stability study;  $n = 3$ ; error bars = RSD

The development of the blister samples is depicted in Figure 5–47 & 48. Every sample of the basic formulation fluctuates around 100 %. The refrigerated sample of the second formulation is relatively stable, slightly above 100 % while the other two storage type resulted in values that are mostly below 100 %.

After twelve weeks, the samples of the basic formulation showed relative contents of 102.6 % ( $n = 1$ ),  $102.5 \pm 2.0$  %,  $94.7 \pm 11.1$  %, whereas the measurements of the second formulation showed values of  $107.0 \pm 1.0$  %,  $87.5 \pm 2.2$  % and  $74.2 \pm 2.0$  %. The basic formulation showed no significant differences to the initial measurement. For the formulation with AP, every post-stability sample showed significant differences ( $p < 0.010$ ) to the initial measurement. Here the refrigerated sample also showed a higher relative concentration, while the other samples had lower concentrations.

Statistical comparison of the different storage conditions was only possible for the 25/60 and 40/75 samples, as two measurements of the refrigerated samples had to be excluded. There was no difference between these two samples ( $p = 0.295$ ). However, the A0 formulation had significantly ( $p < 0.002$ ) different concentrations for each possible pairing. This was unexpected and could be a sign of an insufficiently sealed blister. It is also interesting that the 40/75 sample is lower than the 25/60 sample, as this was not the case for the other types of packaging.

Comparing the associated samples of both formulations showed differences for the 25/60 and 40/75 samples ( $p < 0.035$ ; no evaluation for the fridge samples), in both cases the basic formulation had the higher remaining content.

After the subsequent UV irradiation, the base samples showed relative concentrations of  $100.9 \pm 3.4 \%$ ,  $105.2 \pm 4.8 \%$  and  $106.3 \pm 21.1 \%$ , whereas the formulation with AP had remaining concentrations of  $86.5 \pm 14.6 \%$ ,  $90.9 \pm 24.9 \%$  and  $76.9 \pm 0.6 \%$ . In contrast to the initial quantification only the 40/75 sample of the AP formulation showed an aberration ( $p < 0.001$ ). When the values were compared to the post-stability samples, no differences can be observed ( $p > 0.084$ ).

Lastly, no differences ( $p > 0.138$ ) are identifiable when the associated samples of the formulations are compared with each other.

For the basic formulation, the blister sample had a higher ( $p < 0.008$ ) concentration after twelve weeks than both the bulk and capsule sample for every storage condition (no evaluation for fridge samples). When the second formulation was analysed statistically, the blister samples also had a higher ( $p < 0.013$ ) remaining CBD content than the other two types of packaging at every storage condition. When the same type of analysis was applied to the post-UV samples, differences ( $p < 0.004$ ) were identified between the refrigerated and 25/60 bulk/blister pairings as well as the 25/60 capsule/blister pairing. In each case the blister sample had the higher remaining concentration. When the A0 formulation was evaluated the refrigerated bulk/blister pairing and the 40/75 capsule/blister pairing showed differences ( $p < 0.050$ ). The blister had the higher relative concentration in every pairing. This was expected as the blister is impervious for UV light.

In conclusion it can be said that the blister is expectedly the best of the evaluated types of packaging. However, like the HPMC formulation, several quantifications of the blister samples yielded relative concentrations above 100 %. This is probably a consequence of the heat sealing, which could reduce the amount of residual solvents in the formulation and thus result in a higher CBD content. Therefore, it can be said that the optimal packaging of this formulation would be a capsule enclosed in a blister.

Every post-stability sample of the basic formulation, apart from the refrigerated blister sample, showed a higher remaining CBD content than the corresponding sample of the AP formulation (not every difference is significant). Therefore, It is obvious that the addition of AP is not beneficial for the stability of CBD in an HPMC-AS matrix. It is unlikely that there was a reaction of the AP with the CBD as this should have been visible during the UV-stress test, where a distinctly higher amount of AP was used. However, it could also be possible that the incorporation of AP negatively influences the structure of the HPMC-AS matrix, which could result in an increased vulnerability of the CBD to the applied storage conditions. It also has to be kept in mind that the mode of degradation in this formulation is not completely known. AP can only effectively inhibit the degradation if it follows an oxidative route. For this, the

molecule has to be able to intercept the molecule which reacts with the CBD. However, the interception of these molecules could also be inhibited due to the fact that the CBD and AP are immobilised in the formulation.

### 5.6.2.5 Soluplus® Formulations

As seen with the other formulations, several samples have very high RSDs (up to 37.7%). This also results in a seemingly random progression for some samples (e.g. 40/75 bulk of the AP formulation) This reasons the decision to primarily evaluate the samples after twelve weeks and the subsequent UV irradiation.

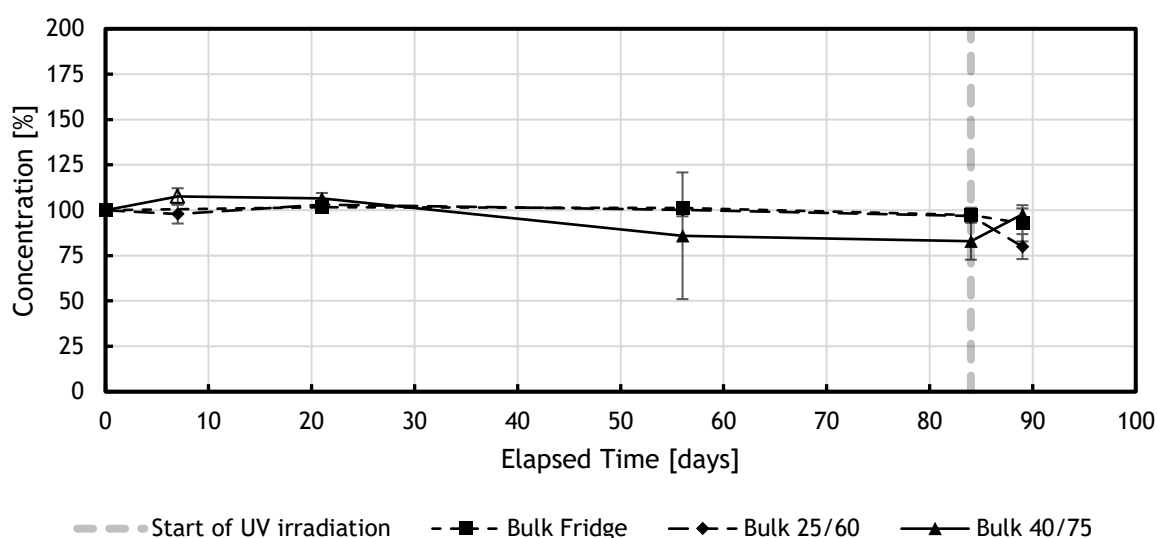


Figure 5-49: Concentration development of the SP + CBD bulk samples over the stability study;  $n = 3$ ; error bars = RSD

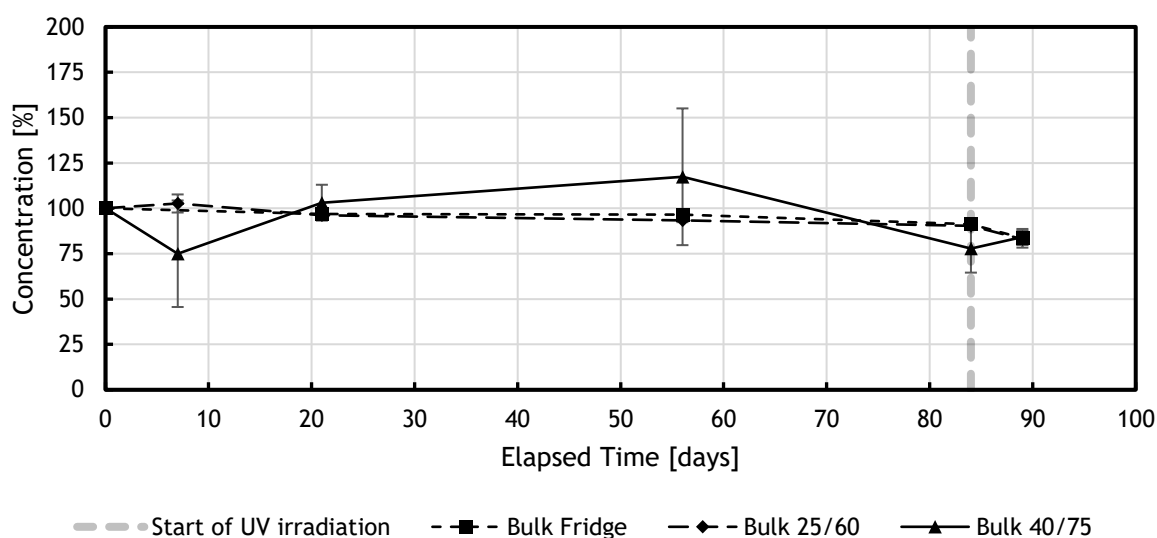


Figure 5-50: Concentration development of the SP + CBD + AP bulk samples over the stability study;  $n = 3$ ; error bars = RSD

Figure 5—49 & 50 show the concentration development of the bulk samples of the SP formulations. Overall, the progression of the samples is relatively stable around 100 %, which signals a good stability in these formulations.

The bulk samples of the basic formulation had relative CBD contents of  $97.4 \pm 3.8 \%$   $96.7 \%$  ( $n = 1$ ) and  $82.9 \pm 8.5 \%$ . For the AP formulation relative concentrations of  $91.2 \pm 0.9 \%$ ,  $90.3 \pm 0.9 \%$  and  $77.8 \pm 10.3 \%$  were measured. Statistical analyses of these values showed that the refrigerated samples of the formulation with AP and both 25/60 samples had lower concentrations than the initial measurement ( $p < 0.004$ ).

Comparing the post-stability bulk samples from different storage conditions with each other showed no differences between any measurements (not possible for basic 25/60 samples ( $n = 1$ )). This was also the case when the corresponding samples of the different formulations were compared ( $p = 0.051$ ).

The subsequent UV irradiation resulted in relative CBD concentrations of  $92.8 \pm 9.2 \%$ ,  $80.0 \pm 5.6 \%$  and  $97.8 \pm 3.1 \%$  for the basic formulation and  $83.6 \pm 3.0 \%$ ,  $83.0 \pm 3.9$  and  $84.2 \pm 3.7 \%$  for the formulation with AP. The 25/60 sample of the basic formulation and every sample of the second formulation were lower than the initial measurement ( $p < 0.025$ ). In comparison to the previous measurement differences were observed for the 25/60 samples of the AP formulation ( $p < 0.034$ ) as well as the 40/75 sample of the basic formulation ( $p = 0.046$ ) and the refrigerated sample of the AO formulation ( $p = 0.014$ ). Interestingly the measured CBD content for the 40/75 sample of the basic formulation was higher after the UV irradiation than after twelve weeks of controlled storage. The formation of new CBD is highly improbable and indicates problems with the analytics or errors during sample preparation.

When the samples of the different formulations were compared with each other, a significantly higher ( $p = 0.008$ ) relative concentration was identified for the 40/75 bulk sample of the basic formulation.

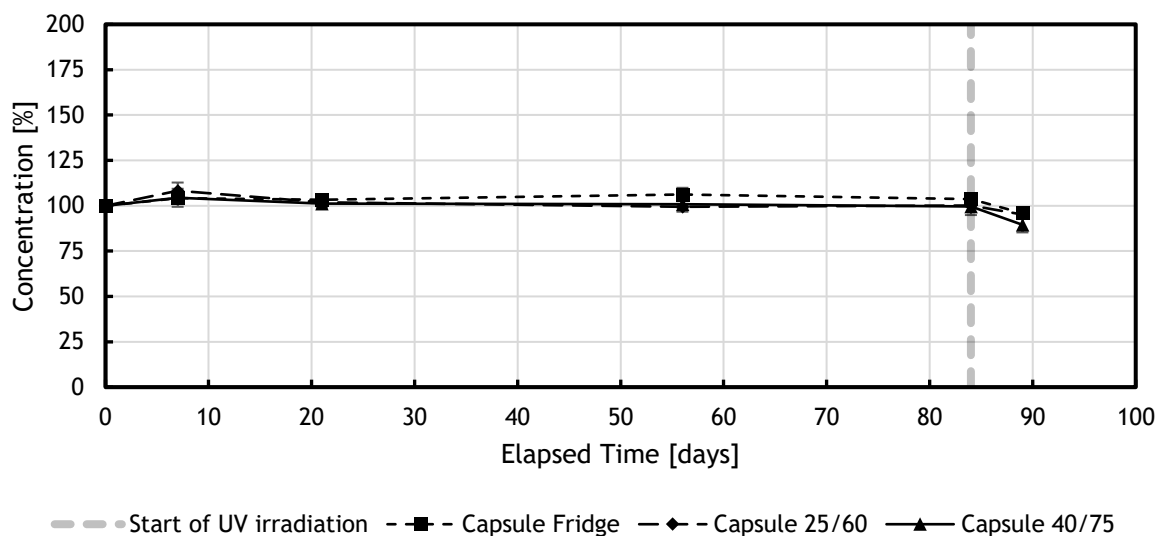


Figure 5–51: Concentration development of the SP + CBD capsule samples over the stability study;  $n = 3$ ; error bars = RSD

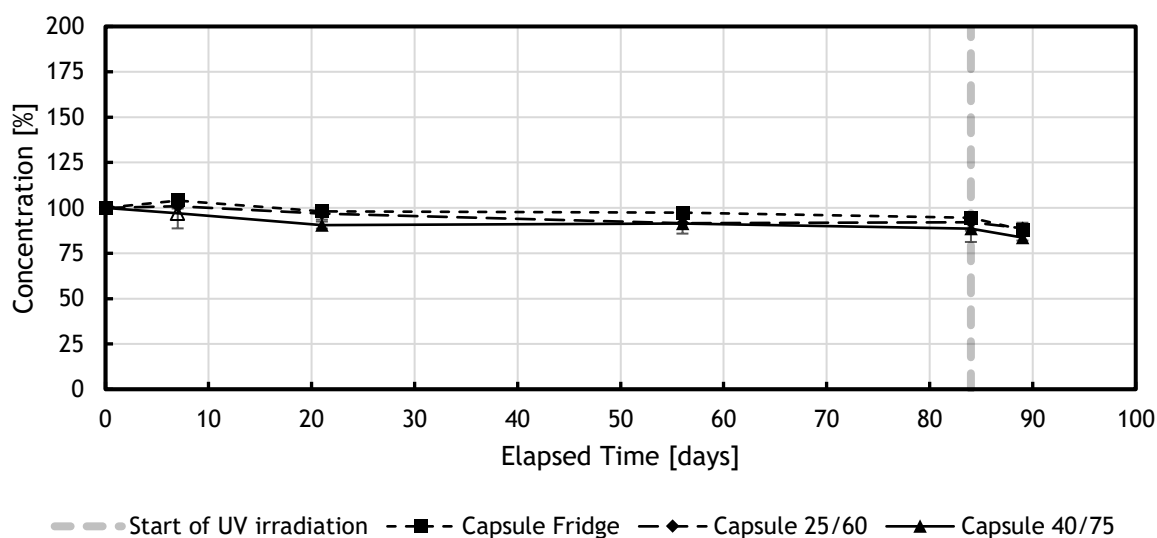


Figure 5–52: Concentration development of the SP + CBD + AP capsule samples over the stability study;  $n = 3$ ; error bars = RSD

The capsule samples of the SP formulations (Figure 5–51 & 52), like its bulk samples, showed a relatively stable progression.

Twelve weeks of controlled storage resulted in relative CBD concentrations of  $103.7 \pm 2.6 \%$ ,  $100.2 \pm 0.6 \%$  and  $99.5 \pm 4.6 \%$  for the basic formulation, whereas the second formulation had relative CBD contents of  $94.6 \pm 0.2 \%$ ,  $92.0 \pm 4.2 \%$  and  $88.5 \pm 6.4 \%$ . Statistical analyses revealed that only the refrigerated sample of the AO formulation had a lower ( $p < 0.001$ ) remaining concentration than before the stability study.

Contrasting the post-stability concentrations of the different storage conditions shows no differences in any pairing ( $p > 0.089$ ). However, the comparison of the corresponding samples

## Twelve-Week Stability of the CBD Matrix Particles

of the two formulations revealed a difference ( $p = 0.025$ ) between the refrigerated samples. Here the basic formulation had the higher post stability concentration.

After the subsequent UV irradiation, the relative concentrations were measured to be  $95.9 \pm 2.9 \%$ ,  $95.2 \pm 1.7 \%$  and  $89.5 \pm 3.7 \%$  for the basic formulation, while the formulation with AP showed remaining contents of  $87.9 \pm 2.9 \%$ ,  $88.7 \pm 2.7 \%$  and  $83.7 \pm 1.4 \%$ . In contrast to the initial CBD content, every sample, except for the refrigerated sample of the basic formulation, had a lower content ( $p < 0.041$ ).

Every sample of the basic formulation sustained a loss of CBD compared to the previous measurement ( $p < 0.043$ ), whereas no difference could be identified for the samples of the second formulation ( $p > 0.055$ ). Nonetheless, two samples (fridge, 25/60) of the second formulation showed significantly lower ( $p < 0.028$ ) relative CBD contents than the corresponding samples of the basic formulation.

When the post stability capsule samples of the SP formulations were compared to the corresponding bulk samples statistically, it could be seen, that there were differences between the 40/75 samples ( $p = 0.040$ ) of the basic formulation as well as the refrigerated samples of the AO formulation ( $p = 0.003$ ). In every pairing, the capsule sample had the higher CBD content, which indicates a protective effect of the capsule. The same type of analysis of the post-UV samples showed no differences for the AP formulation, whereas the 25/60 and 40/75 pairings of the basic formulation were different ( $p < 0.043$ ). Interestingly, the 40/75 capsule sample had a lower value than the corresponding bulk sample, while it was reversed for the 25/60 pairing.

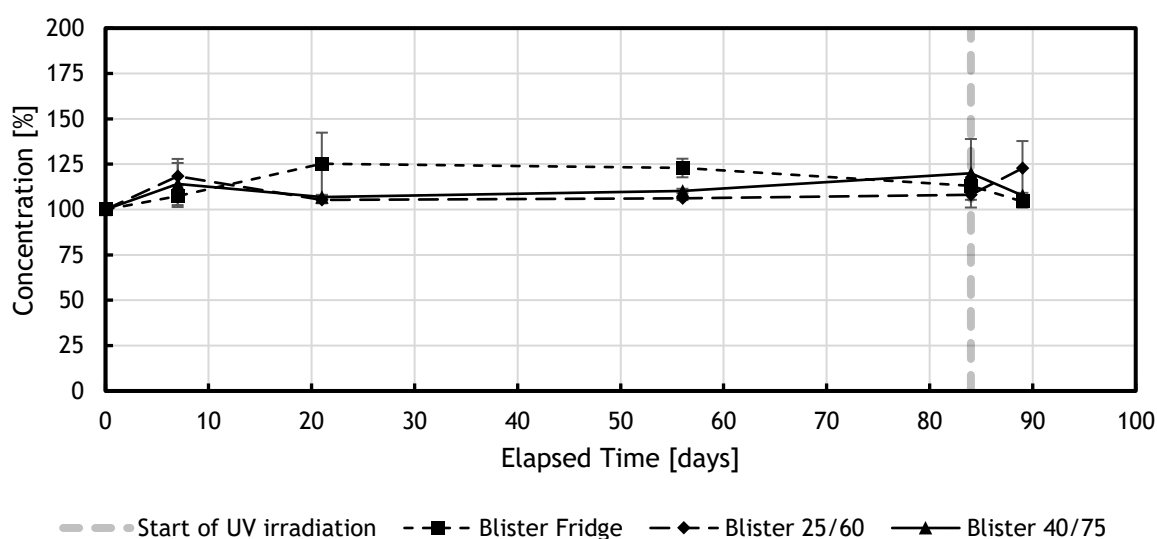


Figure 5–53: Concentration development of the SP + CBD blister samples over the stability study;  $n = 3$ ; error bars = RSD

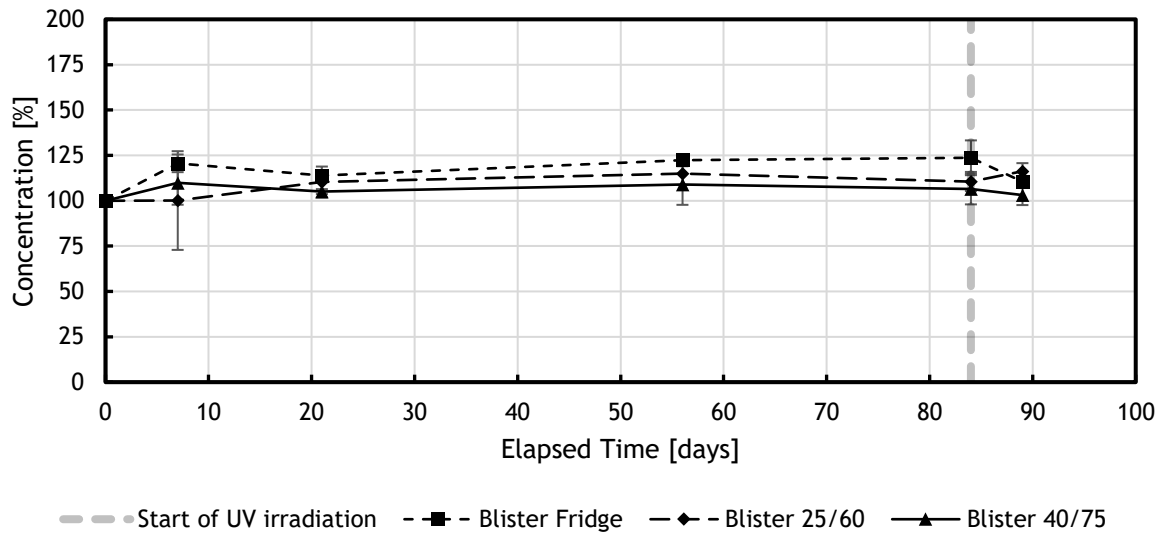


Figure 5–54: Concentration development of the SP + CBD + AP blister samples over the stability study;  $n = 3$ ; error bars = RSD

The development of the blister samples is depicted in Figure 5–53 & 54. Several values are above 100 %, which was already seen with the other matrices. The reason for this is probably the same as with the other formulations: an evaporation of the remaining solvents, which in turn increases the CBD content. This assumption can, again, only be made for the blister samples as the initial filling weight of the individual cups is unknown.

After twelve weeks, the relative concentrations of the base and AP formulation were  $113.1 \pm 5.5 \%$ ,  $108.1 \pm 2.9 \%$  and  $120.0 \pm 22.7 \%$  as well as  $123.7 \pm 11.8 \%$ ,  $110.6 \pm 5.8 \%$  and  $106.4 \pm 9.0 \%$ . In contrast to the initial measurement, the 25/60 sample of the basic formulation was significantly higher ( $p = 0.041$ ).

The comparison of the samples that were stored at different conditions showed no differences ( $p > 0.114$ ). This was also true when the corresponding samples of the different formulations are related to each other.

The UV irradiation of the samples resulted in relative CBD contents of  $104.4 \pm 3.6 \%$ ,  $122.7 \pm 18.3 \%$  and  $107.8 \pm 1.5 \%$  for the basic formulation and  $110.6 \pm 2.1 \%$ ,  $116.3 \pm 5.2 \%$  and  $103.2 \pm 5.7 \%$  for the second formulation. This time, three significant differences ( $p < 0.033$ ), compared to the initial measurement, could be identified. These differences were found for the 40/75 base sample and the refrigerated and 25/60 sample of the AO formulation. Each time the post-UV sample had the higher CBD concentration, which indicates analytical inaccuracies. However, when the post-UV measurements were compared with the twelve-week measurements no pairing showed a difference ( $p > 0.082$ ).

When the different storage conditions were compared among each other, only the 25/60 and 40/75 samples of the AP formulation showed a difference ( $p = 0.043$ ). Like the post-stability

samples, there was no difference ( $p > 0.060$ ) between the CBD concentrations of the corresponding post-UV blister samples of the different formulations.

Comparing the different types of packaging showed that every comparable (25/60 base bulk sample was  $n = 1$ ) post-stability bulk/blister pairing, except for the 40/75 base pairing, had significantly different ( $p < 0.041$ ) CBD concentrations. When the capsule samples were related with the blister samples both 25/60 samples showed differences ( $p < 0.011$ ) as well as the 40/75 samples of the AP formulation ( $p = 0.048$ ). The blister sample had the higher CBD content in every significantly different pairing.

Performing the same analysis for the post-UV samples showed that the 25/60 and 40/75 blister samples of the basic formulation had higher ( $p < 0.018$ ) CBD contents than the corresponding bulk samples. Compared with the capsules, the blister had higher ( $p < 0.034$ ) remaining CBD concentrations after storage in the refrigerator or at 40 °C and 75 % rH. When the second formulation is analysed, the blister samples had higher ( $p < 0.009$ ) relative concentrations than both other types of packaging at all three storage conditions.

Finally, it can be said that the CBD is very stable in a SP matrix stored in darkness. This was the case with and without the AP. However, as every sample of the basic formulation, which was not stored in a blister, had higher relative post stability CBD concentrations than the corresponding sample of the formulation with AP, it can be concluded that the addition of AP slightly reduces the stability of CBD in this type of formulation. This is probably for the same reasons which were hypothesised for the HPMC-AS formulation (see chapter 5.6.2.4).

As seen with the other matrices the packaging in the blisters resulted in an increased CBD content. Therefore, the optimal packaging for an eventual medicine would probably be a capsule enclosed in a blister. As this would reduce the effect of the heat sealing on the formulation.

### *5.6.3 PROPERTY CHANGE OF THE FORMULATIONS OVER TWELVE WEEKS*

The following chapters show the changes of the physicochemical properties of the six formulations as well as their macroscopic changes. Like the previous chapter, three results named in succession are always in the order: refrigerated sample, 25/60 sample and 40/75 sample.

#### *5.6.3.1 HPMC Formulations*

Macroscopic evaluation of the samples did not show any striking differences. However, the samples stored at 25 °C and 60 % rH and 40 °C and 75 % rH exhibited a slightly yellowish, off-white colour, which could indicate degradation of CBD and/or AP. Additionally, it must be mentioned that the formulation stuck to the top foil when the blister was opened and that in



some cups the formulation built comparatively solid plugs. This is probably a result of the heat that was applied during the sealing process. To check if the temperature was responsible for the changes of the formulations, the powder was put on a 180 °C heating plate. Both formulations became sticky and got a darker colour, which proves that the formulation is not fit for this type of blister sealing method.

Table 5–19: Change of physico-chemical properties of the HPMC formulations stored in bulk (top - base; bottom - AO formulation);  $n = 3$ ;  $n = 10$ ; cursive:  $n = 2$ ; values without SD could only be measured once; asterisks show significance to the initial value

HPMC + CBD	Initial value ( $\pm$ SD)	Refrigerated ( $\pm$ SD)	25 °C, 60 % rH ( $\pm$ SD)	40 °C, 75 % rH ( $\pm$ SD)
Density [ $g/cm^3$ ]	1.261 ( $\pm$ 0.015)	1.206 ( $\pm$ 0.011)***	1.221 ( $\pm$ 0.015)***	1.205 ( $\pm$ 0.008)***
Particle Size ( $x_{50}$ ) [ $\mu m$ ]	6.84 ( $\pm$ 0.20)	6.02 ( $\pm$ 0.04)*	7.47 (N/A)	7.90 ( $\pm$ 0.34)**
Span	2.73 ( $\pm$ 0.07)	3.41 ( $\pm$ 0.41)	2.58 (N/A)	2.99 ( $\pm$ 0.86)
Maximum Water Uptake [%]	16.9	16.2	15.8	16.3
HPMC + CBD + AP	Initial value ( $\pm$ SD)	Refrigerated ( $\pm$ SD)	25 °C, 60 % rH ( $\pm$ SD)	40 °C, 75 % rH ( $\pm$ SD)
Density [ $g/cm^3$ ]	1.229 ( $\pm$ 0.006)	1.213 ( $\pm$ 0.008)***	1.205 ( $\pm$ 0.007)***	1.169 ( $\pm$ 0.010)***
Particle Size ( $x_{50}$ ) [ $\mu m$ ]	6.36 ( $\pm$ 0.22)	6.98 ( $\pm$ 0.25)	7.12 ( $\pm$ 0.17)	8.09 ( $\pm$ 0.37)
Span	2.53 ( $\pm$ 0.08)	2.60 ( $\pm$ 0.21)	2.50 ( $\pm$ 0.07)	2.42 ( $\pm$ 0.13)
Maximum Water Uptake [%]	13.9	13.7	11.9	12.3

Table 5–19 shows the change of the physico-chemical properties of the HPMC formulations bulk samples over the course of the stability study. The upper part of the table contains the data for the basic formulation, while the lower half depicts the data for the AO formulation.

Interestingly, every post-stability sample had a slightly lower density than the formulation had after production. This is unexpected as storage at the more stressful conditions could result in a dissolution of some matrix substance from the surface, which could in turn precipitate in pores this would result in an increased density. However, as the changes are very

marginal (max. change: 4.9 %), the differences of the density cannot be considered relevant. The changes could be a result of the small sample volume that was used in the apparatus. A larger volume would enable the measurement of a larger particle collective and thus reduce the measuring error. However, this was not possible due to a limited amount of formulation. Every storage condition led to changes in the  $x_{50}$  values. The reason for this is probably the absorption of water. The water could lead to swelling or induce stickiness or coalescence of the particles. However, the storage could also lead to a reduction of electrostatic charges, which would lead to smaller  $x_{50}$  values. For the basic formulation, the mean particle size of the refrigerated samples was reduced ( $p = 0.019$ ), whereas the harsher conditions led to larger  $x_{50}$  values. Although, the difference was only significant for the 40/75 samples ( $p < 0.009$ ), as it was not possible to generate more than one successful measurement of the 25/60 samples. This could be due to the formation of strong agglomerates, problems with the equipment or a change in the diffraction behaviour of the formulation. Storage of the AO formulation resulted in larger  $x_{50}$  values ( $p < 0.032$ ) for every storage condition. However, none of the post-stability span values was different from the initial measurement ( $p > 0.106$ ).

The capsule samples also showed changes to the mean particle size. The  $x_{50}$  of the refrigerated samples of both formulations was smaller (base:  $5.83 \pm 0.09 \mu\text{m}$ ; AO formulation:  $5.16 \pm 0.37 \mu\text{m}$ ) than initially ( $p < 0.009$ ), whereas the mean particle size of the samples was increased (base: 25/60:  $7.70 \pm 0.31 \mu\text{m}$ ; 40/75:  $8.25 \pm 0.41 \mu\text{m}$ ; AO formulation: 25/60:  $7.15 \pm 0.31 \mu\text{m}$ ; 40/75:  $9.60 \pm 1.04 \mu\text{m}$ ) significantly ( $p < 0.034$ ). There were no changes in the span values ( $p > 0.322$ ). Like the 25/60 bulk sample of the basic formulation, the blister samples only yielded one successful measurement (for each storage condition), while it was not possible to generate any data for the blister samples of the AO formulation. The reasons for these problems are probably the changes that were observed when the blister was opened: a formation of a sturdy plug which could not be dispersed and was too large to be evaluated. The successful measurements showed distinctly larger (base:  $9.44 \mu\text{m}$ ,  $8.11 \mu\text{m}$  and  $9.49 \mu\text{m}$ )  $x_{50}$  values than the initial measurement. Two of the span values of the blister samples are also clearly higher than initially (27.6907, 3.1788 and 27.2645). This shows that the  $x_{90}$  value of the samples was drastically higher than before the stability study or packaging in the blister, which strengthens the assumption that the sealing procedure of the blister had an influence on the formulation. The results suggest that the packaging in a capsule does not provide protection from the storage conditions. However, as packaging in a blister also results in problems, it should be considered to package the filled capsules in a blister.

Post stability DVS analyses of the basic formulation showed maximum water uptakes of 16.2 %, 15.8 % and 16.3 % when it was stored in bulk. The capsule samples had uptakes of 15.3 %, 15.3 % and 15.3 %.

16.1 % and 15.2 % whereas the blister samples had uptakes of 16.8 %, 17.8 % and 15.8 %. In comparison to the initial value (16.9 %) every sample but the 25/60 blister sample had a lower maximum water uptake. The biggest change from the initial value was -1.7 % of total water uptake. This is a relative change of > 10 %. Which could be a result of an already increased water content. However, it is difficult to evaluate if the observed changes are a real change of the maximum water uptake or if they are a result from a reduced uptake velocity, as the diagrams of the different samples look very similar. A distinctly reduced uptake velocity was observable for a single sample of the SP formulations (see chapter 5.6.3.3). A reduced adsorption velocity would result in a premature termination of the humidity steps which results in a lower maximum value. A reduction in the uptake velocity could be a result of a reduced surface area in the sample. The available surface could be reduced by a tight agglomeration (or increase of particle size) during the storage or changes to the surface itself, like a decrease of the surface roughness. However, a more comprehensive evaluation is not possible because every sample could only be analysed once as a DVS measurement is a lengthy procedure.

Overall, no trend was observable for any packaging or storage condition. None of the formulations showed any events, like a recrystallisation, during analysis. This was expected, as the initial sample also showed no events

After the stability study, the bulk samples of the AP formulation showed maximum water uptakes of 13.7 %, 11.9 % and 12.3 % whereas the analyses of the capsule samples yielded values of 13.8 %, 12.0 % and 9.0 %. The blister samples had uptakes of 13.6 %, 14.4 % and 13.4 %. The 25/60 and 40/75 bulk and capsule samples showed a relative deviation of > 10 %. Additionally, a trend could be observed for the capsule samples. These samples show increasing deviations from the initial value of 13.9 % with increasing stress, applied by the storage conditions. Interestingly this trend was not observable for the bulk samples, although there were no macroscopic differences, like a different amount of agglomerates. However, changes to the adsorption velocity due to changes of the surface or an already increased water content could still explain this difference. Additionally, it was seen that none of the samples showed events during analysis, which, again, was expected.

Only the 40/75 bulk samples were evaluated with XRPD, as these had the highest probability of a recrystallisation of CBD. The reason for this is the absence of any protective packaging as well as the appliance of the harshest storage conditions. The diffractogram of the HPMC formulations with AP is shown in Figure 5—55. The diffractogram of the basic formulation is not shown, as it shows no signs of recrystallisation. The AO formulation has a very small

## Twelve-Week Stability of the CBD Matrix Particles

peak at about 32 2θ, which could be a sign of a beginning recrystallisation of either the CBD or the AP.

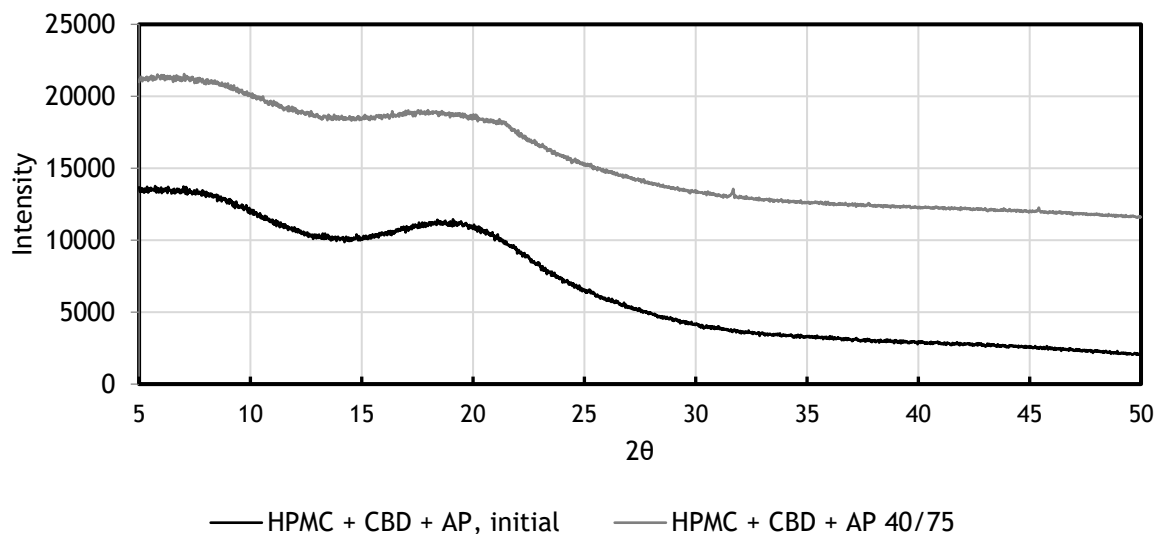


Figure 5—55: X-Ray diffractogram of the HPMC + CBD + AP formulation; 40/75 sample shifted by + 10000 intensity

The DSC diagrams of both formulations after the stability study and each storage/package combination show no signs of crystallinity. An exemplary diagram (basic formulation, capsule, 40/75) is shown in Figure 5—56.

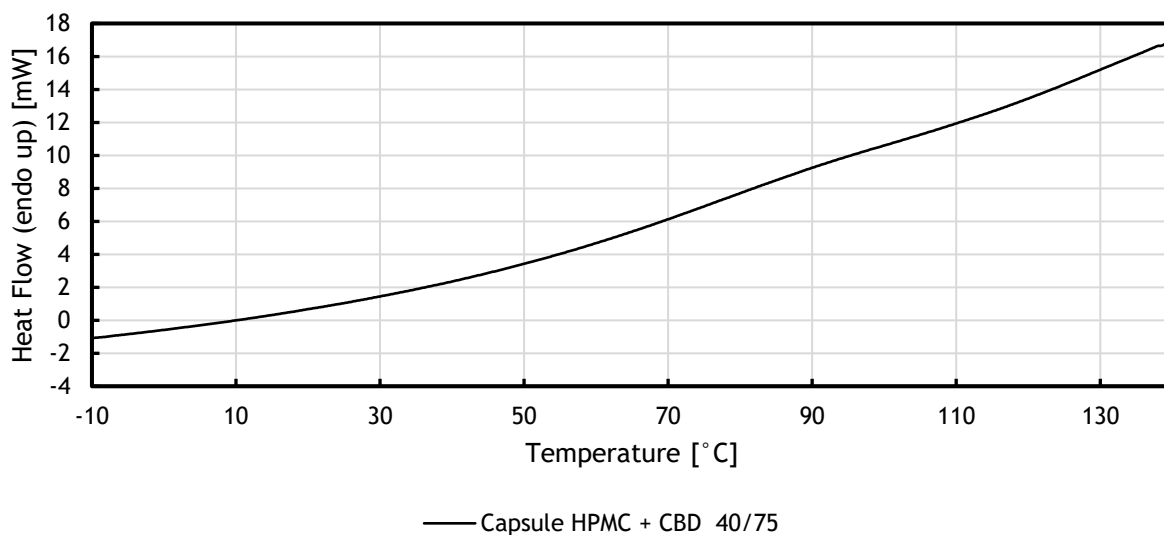


Figure 5—56: DSC diagram of the HPMC + CBD formulation; 40/75 capsule sample

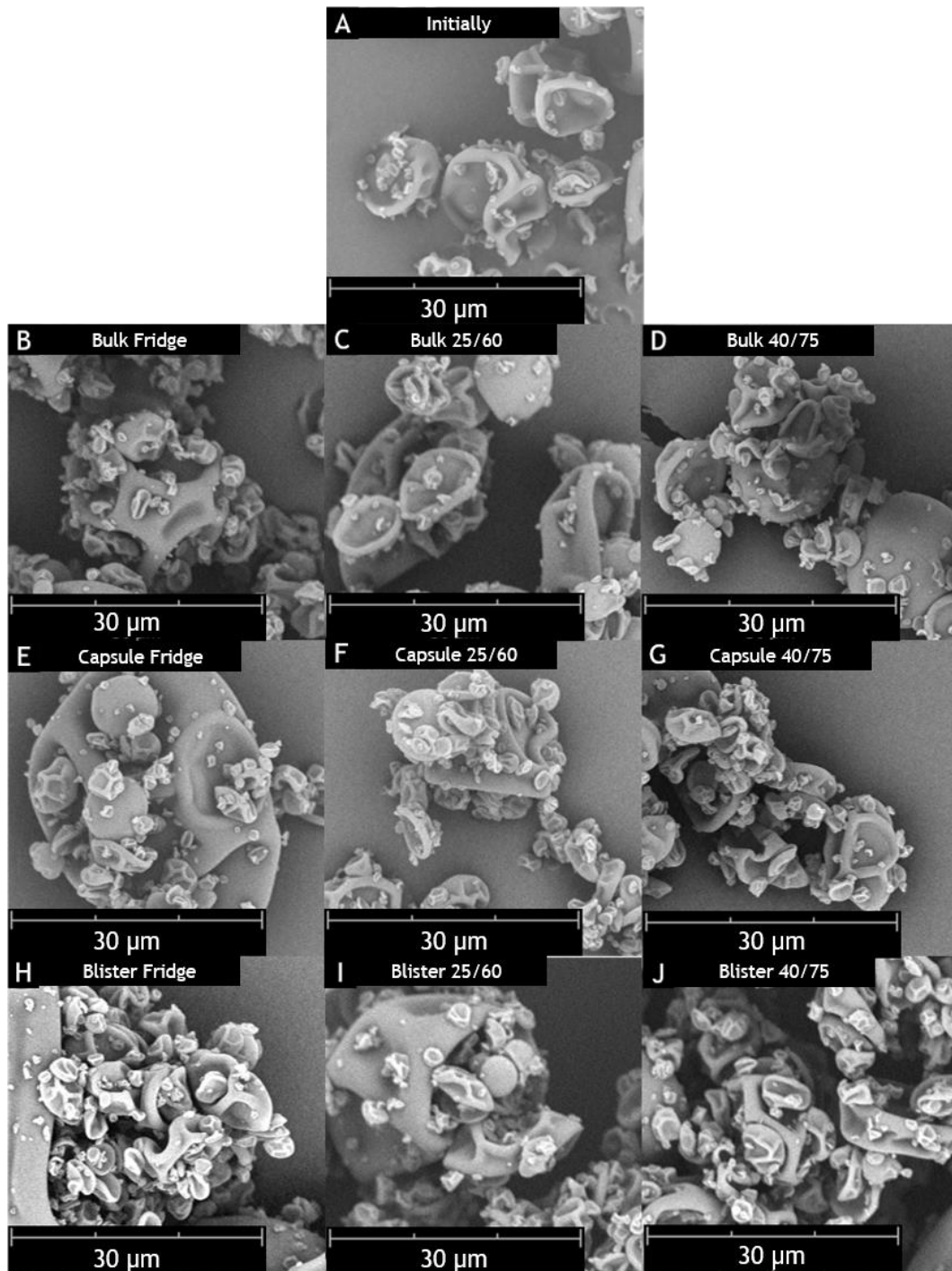


Figure 5—57: SEM images of the HPMC + CBD formulation, 2500x magnification; A - Initial; B-D: Bulk; E-G: Capsule; H-J: Blister; B, E, H: Fridge; C, F, I: 25 °C & 60 % rH; D, G, J: 40 °C & 75 % rH

Figure 5—57 shows SEM images of the HPMC basic formulation before and after the Twelve-Week stability study. There are no striking differences before and after the study. The particles exhibit the shape of a deflated sphere and a relatively broad particle size distribution is visible. The blister samples (Figure 5—57 H-J) seem to be more compacted than the other samples, which makes sense, as the blisters were filled volumetrically by applying a vacuum.

This was also confirmed by macroscopic inspection of the formulation from the blisters, as the powder was only removable from the blister in relatively large agglomerates.

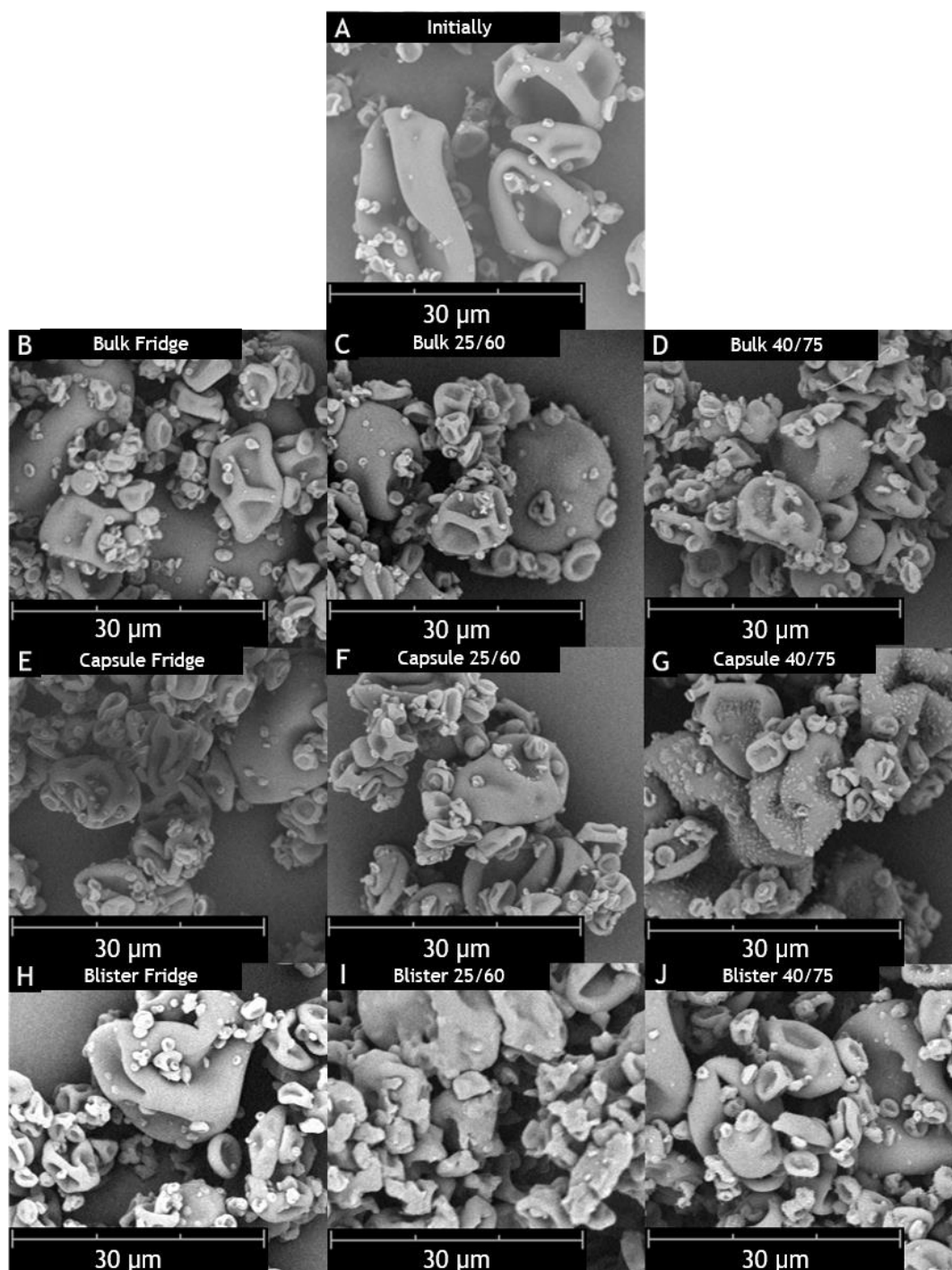


Figure 5—58: SEM images of the HPMC + CBD formulation, 2500x magnification; A - Initial; B-D: Bulk; E-G: Capsule; H-J: Blister; B, E, H: Fridge; C, F, I: 25 °C & 60 % rH; D, G, J: 40 °C & 75 % rH

Figure 5—58 shows SEM pictures of the HPMC + CBD + AP formulation. The samples also show the shape of a deflated sphere. The 25/60 bulk and the 40/75 bulk and capsule samples

(Figure 5—58 C, D, G) look like there are some crystalline formations on the surface. Interestingly, this seems to be further developed for the sample stored in the capsule. However, it should not be forgotten that SEM pictures do not depict the entirety of the sample and that these observations are dependent on the portion of the sample that was adhered to the sample holder. The 25/60 blister sample (Figure 5—58 I) shows some changes on the surface. These look as if the formulation began to melt before solidifying again, which could be explained by the sealing method of the blister, for which 180 °C was applied.

The compiled data suggests that both formulations were relatively stable during the stability study. However, evaluation of the SEM pictures shows that the AP formulation underwent some changes during the storage. This suggests that the basic formulation has a superior stability to the AO formulation.

### 5.6.3.2 HPMC-AS Formulations

Apart from the 40/75 bulk and capsule samples of the AP formulation, the macroscopic evaluation of the samples shows little to no change after the stability study. These samples formed a stable slightly brownish plugs that had the exterior of a powdery formulation. However, these plugs could only be broken down by applying considerable force with a spatula. This hardness will influence the aerodynamic behaviour as well as the ability to perform PSD measurements. Like the HPMC formulations, the blister samples were fused together and stuck to the top foil when the blister was opened. These formulations were also heated to 180 °C to evaluate if the heat that was applied during the sealing method was responsible for the agglomeration. Both formulations melted, which proves that they are not suitable for heat sealed blisters.

Table 5—20: Change of physico-chemical properties of the HPMC-AS formulations stored in bulk (top - base; bottom - AO formulation);  $n = 3$ ;  $\underline{n = 10}$ ; *cursive*:  $n = 2$ ; values without SD could only be measured once; asterisks show significance to the initial value

HPMC-AS + CBD	Initial value (± SD)	Refrigerated (± SD)	25 °C, 60 % rH (± SD)	40 °C, 75 % rH (± SD)
Density [g/cm <sup>3</sup> ]	1.231 (± 0.009)	1.199 (± 0.014)***	1.238 (± 0.009)	1.210 (± 0.009)***
Particle Size ( $X_{50}$ ) [µm]	5.00 (± 0.27)	5.83 (± 0.09)**	6.39 (± 0.06)*	6.10 (± 0.16)**
Span	3.27 (± 0.05)	2.82 (± 0.13)**	18.61 (± 12.51)	3.22 (± 0.44)
Maximum Water Uptake [%]	7.2	6.7	7.0	5.8

## Twelve-Week Stability of the CBD Matrix Particles

<i>HPMC-AS + CBD + AP</i>	Initial value (± SD)	Refrigerated (± SD)	25 °C, 60 % rH (± SD)	40 °C, 75 % rH (± SD)
<i>Density [g/cm<sup>3</sup>]</i>	1.228 (± 0.004)	1.202 (± 0.017) <sup>***</sup>	1.202 (± 0.009) <sup>***</sup>	1.233 (± 0.003) <sup>**</sup>
<i>Particle Size (x<sub>50</sub>) [µm]</i>	4.48 (± 0.07)	5.16 (± 0.37)	5.05 (± 0.22) <sup>*</sup>	N/A
<i>Span</i>	2.94 (± 0.02)	15.24 (± 21.20)	3.25 (± 0.88)	N/A
<i>Maximum Water Uptake [%]</i>	6.2	6.7	7.2	10.1

Table 5—20 shows the change of the physico-chemical properties of the bulk samples of the HPMC-AS formulations over the course of the stability study. The upper part of the table contains the data for the basic formulation, while the lower half depicts the data for the A0 formulation.

As seen with the previous formulations, the post-stability densities of both formulations were slightly different from the respective initial measurements. However, these differences cannot be considered relevant due to the low changes (max. change 2.6 %).

The  $x_{50}$  values of both formulations changed during the stability study. For the basic formulation, every storage condition resulted in larger ( $p < 0.013$ ) mean particle sizes. The 40/75 samples of the second formulation were not analysable (N/A). The reason for this was probably the aforementioned formation of a sturdy plug. Both the refrigerated and 25/60 samples of the basic formulation had higher  $x_{50}$  values than initially. However, only the difference between the 25/60 sample and the initial measurement was significant ( $p = 0.012$ ). Except for the basic formulation stored under refrigeration, the post-stability span values were not different from the initial values. The refrigerated base sample had a lower span value ( $p = 0.005$ ), which shows a smaller  $x_{90}$  and could be a result of reduced electrostatic charges, which result in a lower amount of tightly agglomerated particles.

The capsule samples of the basic formulation showed larger  $x_{50}$  values ( $5.16 \pm 0.37 \mu\text{m}$ ,  $5.41 \pm 0.55 \mu\text{m}$  and  $6.30 \pm 0.44 \mu\text{m}$ ), although only the 40/75 sample was significantly different from the initial measurement ( $p = 0.001$ ). Of the second formulation, both the refrigerated  $5.16 \pm 0.37 \mu\text{m}$ ,  $p = 0.087$ ) and 25/60 samples ( $7.15 \pm 0.23 \mu\text{m}$ ,  $p = 0.000$ ) were larger than the initial measurement. Like with the bulk samples, analysis of the 40/75 samples was not possible, for the same reasons. As seen with the HPMC formulations, it was not possible to analyse multiple blister samples after any storage condition. In this case, however, only the



25/60 samples yielded one successful measurement, which had a higher  $x_{50}$  than initially (6.90  $\mu\text{m}$ ). None of the measurements showed a span value, which was significantly different from the initial span ( $p > 0.421$ ).

The PSD results suggest that packaging in a capsule is insufficient to protect the formulation from the environment and that the capsules should be further packaged in a blister. As the heat-sealed blister had an influence on the formulation, usage of a capsule is mandatory when this type of blister is used.

After twelve weeks of storage, the bulk samples showed maximum water uptake values of 6.7 %, 7.0 % and 5.8 %. Analyses of the capsule samples yielded uptakes of 6.6 %, 6.8 % and 5.8 % while the blister samples had values of 6.3 %, 6.4 % and 5.7 %. Compared to the initial measurement (7.2 %) or the pure polymer (10.3 %) every post-stability sample showed a lower value, which could be result of an already increased water content in the formulation due to the storage. However, it was expected that the blister prevents absorption during the storage time. It is possible that the blister samples were not tightly sealed or that the sealing process had an influence on the water sorption behaviour. The biggest difference was seen with the 40/75 blister sample (1.5 %). Every 40/75 sample had a relative aberration of more than 19 % from the initial measurement. It could be observed that the maximum water uptake of the blister samples was always lower than that of the other two types of packaging. This could be due to the changes to the formulation, which were probably induced by the sealing method of the blister. Again, these samples were compacted and partly fused with the top foil. This could have resulted in changes to the surface or inhibit the water molecules from accessing the surface of the particles in the middle of the agglomerates. This in turn could lead to a decreased adsorption velocity and therefore a premature termination of the humidity steps. Again, no sample showed recrystallisation or any other event during its respective measurement.

DVS measurements of the second formulation resulted in uptake values of 6.7 %, 7.2 % and 10.1 % for the bulk samples, while values of 6.6 %, 6.5 % and 7.2 % were identified for the capsule samples. The blister samples showed maximum water uptakes of 6.4 %, 6.5 % and 6.1 %. Every sample, apart from the 40/75 blister sample, had a higher value than initially (6.2 %). Especially the distinctly higher value of the 40/75 bulk sample was unexpected. This is interesting, as every sample of the basic formulation exclusively had lower values, probably due to an already increased water content after the stability study. This indicates an influence of the AP on the changes to the adsorption behaviour during the twelve weeks. The bulk samples showed a trend to higher uptakes with increasing harshness of the storage conditions. This trend was not visible for the capsule samples. However, when the two types of packaging

awe compared, the capsule samples showed lower values at each storage condition, which indicates protective effects of the capsule. Similar to the bulk samples, the 40/75 sample showed the highest difference to the initial measurement. This was expected as the samples formed a stable plug. However, it is interesting that these samples adsorbed more water. It was expected that the compaction of the sample would lead to lower water uptakes, as the accessibility of the inner particles' surface could be reduced. The blister samples were also compacted, probably a result of the filling and sealing methods. However, the maximum water uptake of these samples was comparatively stable. This, on the one hand, shows that the type of compaction/agglomeration of the respective samples was different and on the other hand that the blister was better at protecting than the capsule, especially at 40 °C and 75 % rH, which was expected.

Data suggests that storage, especially at 40 °C and 75 % rH, had effects on the water sorption behaviour of this formulation. Additionally, it could be seen that no sample showed signs of recrystallisation or any other event during the analysis.

Like with the HPMC formulations, only a few samples were reevaluated with XRPD. In this case, 25/60 and 40/75 bulk samples of the basic formulation and a 40/75 bulk sample of the formulation with AP were analysed. The basic formulation did not show any signs of crystallinity. However, the A0 formulation developed some small peaks between 17 and 25  $2\theta$  (Figure 5–59). This is a sign of a beginning recrystallisation of either CBD or AP. This suggests that either the AP itself recrystallises or that it induces a recrystallisation of the CBD, either due to its presence, or due to a shift in the ratio of matrix and API. Interestingly, no peak is one of the main peaks that were visible in the diffractograms of the pure substances. This could mean that the recrystallising substance recrystallizes in an unknown modification.

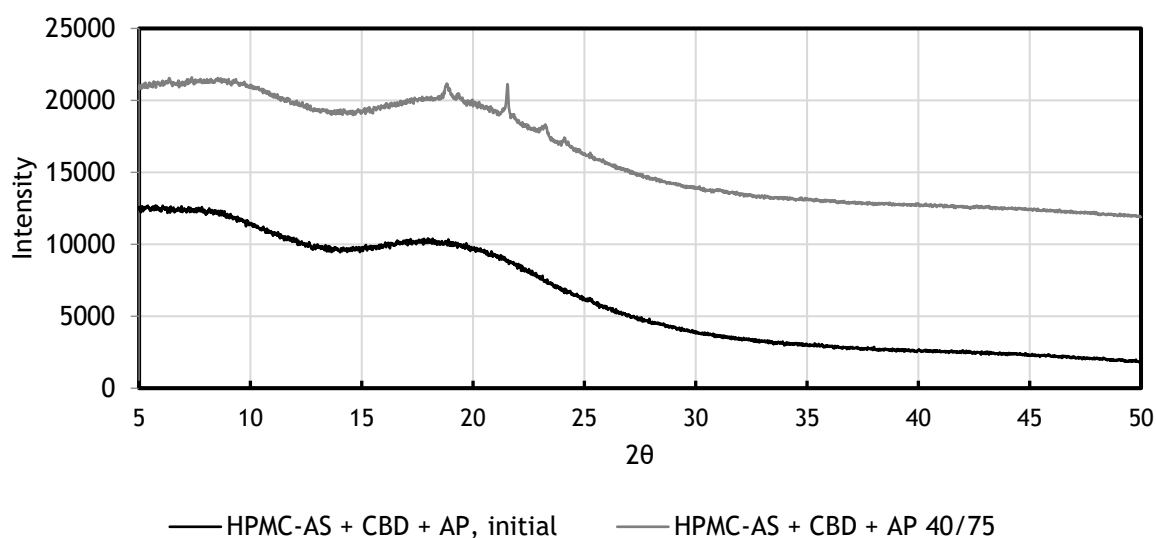


Figure 5–59: X-Ray diffractogram of the HPMC-AS + CBD + AP formulation; 40/75 sample shifted by +10000 intensity

The observations about the beginning recrystallisation in the HPMC-AS + CBD + AP formulation were confirmed by DSC (Figure 5—60). The diagram showed a very small peak just below 70 °C. The used instrument measured the melting point of CBD to be 70.12 °C (see 3.1.1), which suggests the observed peak to derive from recrystallisation of the API.

The HPMC-AS + CBD + AP sample, stored at 25 °C and 60 % rH, had no peak in the diagram. However, the diagram is not as smooth as the initial measurement in the area between 60 and 75 °C. The capsule and blister samples showed no changes in the DSC diagrams. This suggests that these types of packaging can inhibit recrystallisation of the components in an HPMC-AS formulation.

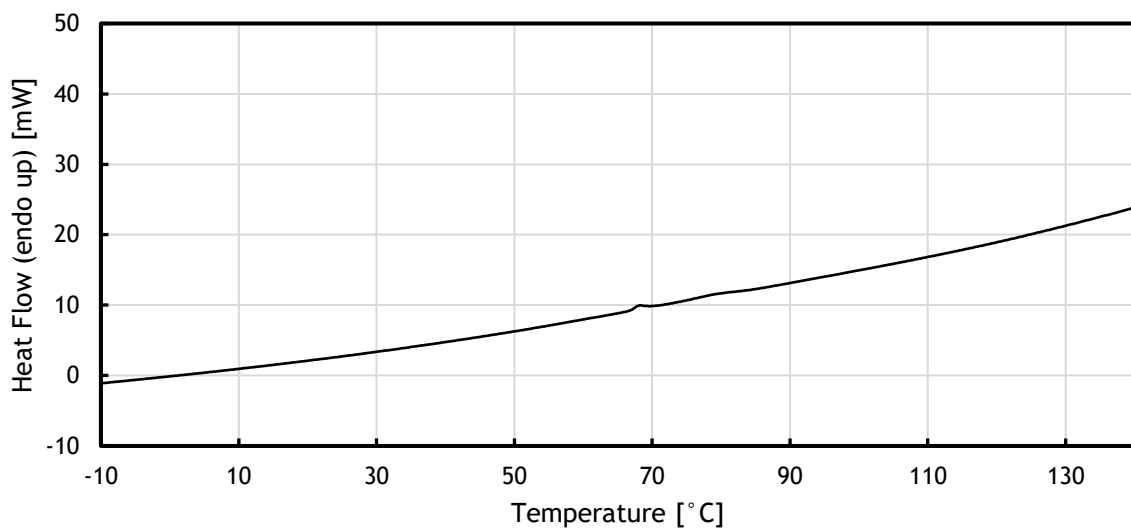


Figure 5—60: DSC diagram of the HPMC-AS + CBD + AP formulation stored in bulk at 40 °C and 75 % rH

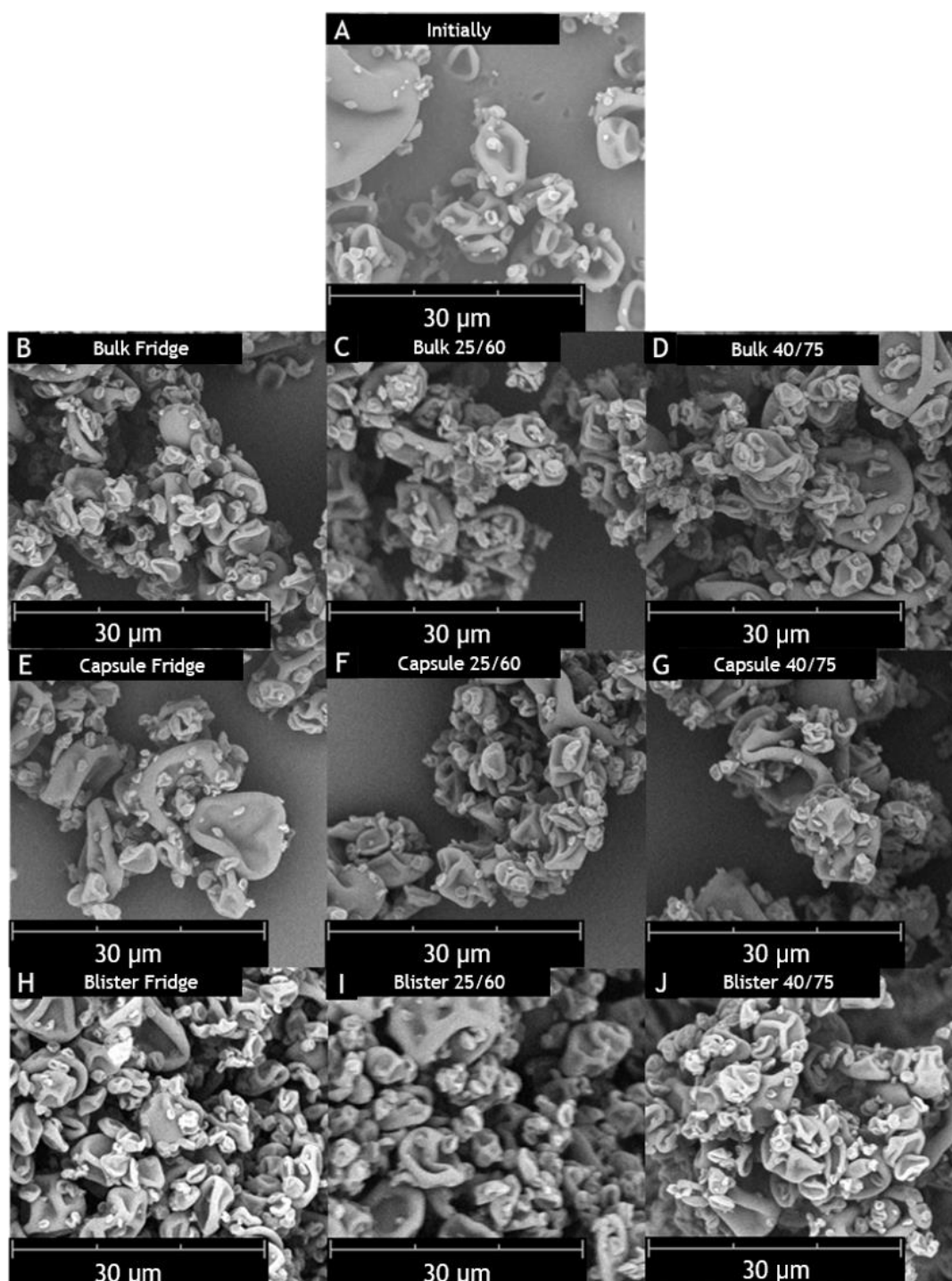


Figure 5—61: SEM images of the HPMC-AS + CBD formulation, 2500x magnification; A - Initial; B-D: Bulk; E-G: Capsule; H-J: Blister; B, E, H: Fridge; C, F, I: 25 °C & 60 % rH; D, G, J: 40 °C & 75 % rH

Figure 5—61 shows SEM images of the HPMC-AS + CBD formulation before and after the stability study. There was no change in the exterior of the particles, regardless of packaging or storage conditions. Every image shows particles of similar sizes with a relatively broad PSD, while they exhibit the shape of a deflated sphere.

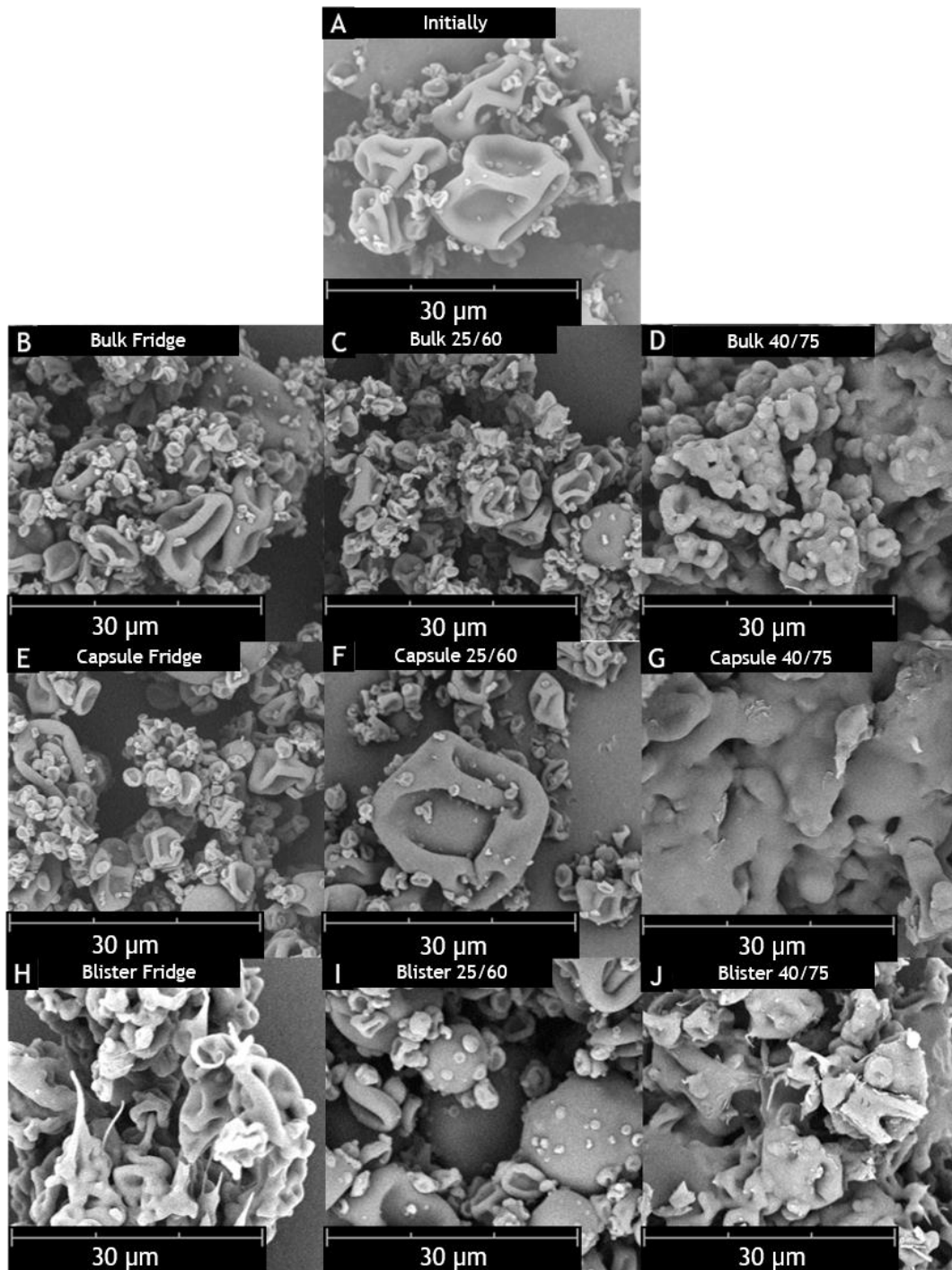


Figure 5—62: SEM images of the HPMC-AS + CBD + AP formulation, 2500x magnification; A - Initial; B-D: Bulk; E-G: Capsule; H-J: Blister; B, E, H: Fridge; C, F, I: 25 °C & 60 % rH; D, G, J: 40 °C & 75 % rH

Figure 5—62 shows the SEM pictures of the HPMC-AS + CBD + AP formulation before and after the stability study. The samples stored in bulk or a in capsule look similar to the initial sample when stored under refrigeration or at 25 °C and 60 % rH (Figure 5—62 B, C, E, F). After storage at 40 °C and 75 % rH the particles look as if they had been melted together (Figure 5—62 D,G). As the storage conditions are below the melting points of the components, it can be suspected that this happened because of the increased humidity. This, for example, could

happen due to a dissolution of the particles surfaces, which then coalesce, resulting in the visible exterior.

The samples stored in the blister (Figure 5—62 H-J), especially after refrigerated storage or storage at 40 °C and 75 % rH, also look as if some parts had melted, perhaps due to the sealing process of the blisters. This is consistent with the macroscopic evaluation of the blister samples, as a large fraction of the powder stuck to the top foil when the blister was opened. The sample stored at 25 °C and 60 % rH looks relatively similar to the initial sample. However, some smaller particles seem to have been fused into larger ones. This suggests that the particles remain relatively consistent if they are not changed during the sealing process.

Data show that the formulation with AP is more susceptible to the storage conditions. This is especially obvious when the 40/75 samples are compared with each other. The AP formulation was no longer analysable with the HELOS device, as it formed a solid plug, and the SEM pictures show distinct changes to the images of the formulation before the stability study. Additionally, the formulation showed signs of recrystallisation. All of this was not observable for the basic formulation. Therefore, the basic formulation has to be favoured from a physicochemical stability standpoint.

### *5.6.3.3 SP Formulations*

The macroscopic evaluation of the SP formulations showed striking differences between the 40/75 samples of both formulations and the respective formulation after production. Similar to the HPMC-AS AP formulation, the basic formulation formed a sturdy powder plug with an off-white colour. This powder plug could only be broken up by applying a considerable amount of pressure with a spatula. The second formulation underwent an even more obvious transformation. The formulation had no similarity with a powder anymore. The bulk samples formed solid, amber-coloured droplets that adhered to the surface of the Eppendorf tube. This could be a result of significant a water uptake, which resulted in the formation of resin-like drops. The samples that were stored at the milder conditions underwent no colour or obvious macroscopic changes. In the capsule, almost the total amount of the formulation coalesced and formed one large plug that looked similar to amber. This plug was very sturdy and could only be broken by applying significant pressure with a hard tool. Breakage of this plug indicated that it was no agglomeration of powdery components but one large solid. These changes will have a significant influence on the aerodynamic behaviour and the PSD measurements.

Additionally, both formulations 25/60 samples formed agglomerates and show a yellowish off-white colour after twelve weeks. There were no obvious changes to the formulations that were stored in refrigerator.

Like the other formulations, opening of the blister revealed that a large fraction of the formulation was fused with the top foil, which indicates that the heat-sealing of the blisters is not suitable for the SP formulations. This was, again, proven by putting the formulations on a 180 °C heating plate. Both formulations immediately melted, like the HPMC-AS formulations, which shows that they cannot be packaged in heat sealed blisters.

Table 5—21: Change of physico-chemical properties of the SP formulations stored in bulk (top - base; bottom - A0 formulation);  $n = 3$ ;  $n = 10$ ; cursive:  $n = 2$ ; values without SD could only be measured once; asterisks show significance

<i>SP + CBD</i>	<i>Initial value (± SD)</i>	<i>Refrigerated (± SD)</i>	<i>25 °C, 60 % rH (± SD)</i>	<i>40 °C, 75 % rH (± SD)</i>
<i>Density [g/cm<sup>3</sup>]</i>	<u>1.165</u> (± 0.007)	<u>1.141</u> (± 0.005)***	<u>1.142</u> (± 0.009)***	<u>1.130</u> (± 0.007)***
<i>Particle Size (x<sub>50</sub>) [µm]</i>	5.65 (± 0.11)	6.58 (± 0.59)	7.47 (N/A)	N/A
<i>Span</i>	2.27 (± 0.04)	13.08 (± 18.72)	33.45 (N/A)	N/A
<i>Maximum Water Uptake [%]</i>	16.5	14.9	14.0	13.5
<i>SP + CBD + AP</i>	<i>Initial value (± SD)</i>	<i>Refrigerated (± SD)</i>	<i>25 °C, 60 % rH (± SD)</i>	<i>40 °C, 75 % rH (± SD)</i>
<i>Density [g/cm<sup>3</sup>]</i>	<u>1.333</u> (± 0.014)	<u>1.134</u> (± 0.011)***	<u>1.130</u> (± 0.008)***	<u>1.127</u> (± 0.007)***
<i>Particle Size (x<sub>50</sub>) [µm]</i>	6.59 (± 0.17)	7.59 (N/A)	N/A	N/A
<i>Span</i>	2.01 (± 0.03)	14.79 (N/A)	N/A	N/A
<i>Maximum Water Uptake [%]</i>	17.0	10.9	10.8	9.3

Table 5—21 shows the change of the physico-chemical properties of the bulk samples of the SP formulations over the course of the stability study. The upper part of the table contains the data for the basic formulation, while the lower half depicts the data for the A0 formulation. As with the HPMC and HPMC-AS formulations, the density of the basic formulation changed marginally (max. change: 3.0 %) and is therefore not considered relevant. The second formulation had distinctly larger changes to the density. In this case every storage condition resulted in a lower density (-14.9 %, -15.2 % and -15.4 %). This is very interesting, especially in

the case of the 40/75 sample, as the macroscopic changes of this sample were considerable. Due to these changes, it was expected that the density of this sample would be considerably higher. However, as the initial measurement of this formulation resulted in an unexpectedly high density, it is possible that the initial measurement is faulty and that the true changes are possibly more comparable to that of the other formulations.

Changes in the PSD could be observed for both formulations. However, only the refrigerated samples of the basic formulation yielded multiple successful measurements with insignificantly different mean values ( $p = 0.113$ ). The lack of successful measurements could be a result of agglomeration or changes in the diffraction behaviour of the sample. The singular post-stability PSD analysis showed larger  $x_{50}$  values and every span value was distinctly larger than initially, which is a result of distinctly increased  $x_{90}$  values. This strengthens the assumption that the generation of successful measurements was hampered by agglomeration of the formulations.

Data generation was more successful with the capsule samples. The refrigerated and 25/60 samples of both formulations yielded at least two successful measurements. Both determined  $x_{50}$  values for the basic formulation (fridge:  $6.58 \pm 0.30 \mu\text{m}$ ; 25/60:  $6.38 \pm 0.35 \mu\text{m}$ ) were larger than initially ( $p < 0.025$ ). In contrast, both values for the AP formulation (fridge ( $n = 2$ ):  $8.02 \pm 0.96 \mu\text{m}$ ; 25/60:  $8.05 \pm 3.78 \mu\text{m}$ ) showed an insignificant ( $p > 0.285$ ) difference. None of the calculated span values showed a significantly ( $p > 0.082$ ) different value than before the stability study. The blister samples could not be evaluated with laser diffraction, probably due to the agglomeration or changes that were a result of the sealing procedure.

DVS analyses of the bulk samples of the basic formulation yielded maximum water uptake values of 14.9 %, 14.0 % and 13.5 %. The corresponding values for the capsule samples were 14.9 %, 14.0 % and 13.6 % whereas the blister samples showed values of 16.1 %, 15.8 % and 14.0 %. In comparison to the initial measurement of 16.5 % (or the pure polymer 21.6 %) every value was lower, which could be a result of an increased water content due to the storage. The bulk and capsule samples behaved similarly while the values of the blister samples are closer to the initial measurement. The particles of every sample (including the pure polymer) stuck together after the respective analysis, which shows a distinct susceptibility of SP to increased humidities, which was expected due to the purpose of the polymer (see chapter 5.5.1). As mentioned before the 40/75 bulk and capsule samples of this formulation formed a stable plug during the twelve weeks. Therefore, it was expected that these values would show a lower water uptake, due to the reduced accessibility of the inner particles, than initially. It is also visible that the negative effect is dependent on the harshness of the storage conditions as there was a clear trend from the refrigerated samples to the 25/60 samples and further



to the 40/75 samples for every type of packaging. These results show that the capsule is not providing sufficient protection from the environment for this formulation. It is possible that the blister samples were not closed completely, as the trend was also visible. The presence of this trend for the blister samples prevents a statement on the effect of the filling and sealing method of these samples, as it would normally be expected that these samples show a similar behaviour. The absence of events during the analyses is consistent with expectations, as the initial sample also showed no events.

The bulk samples of the formulation with AP showed water uptakes of 10.9 %, 10.8 % and 9.3 % whereas the capsule samples had values of 10.7 %, 10.6 % and 2.6 %. For the blister samples they were 11.6 %, 11.9 % and 11.5 %. Every sample had a distinctly lower water uptake than the initial measurement (min. change: -5.1 %), probably due to an already increased water content. A trend, similar to the corresponding samples of the basic formulation is observable for the bulk and capsule samples. It must be mentioned, that the 40/75 capsule sample showed an incredibly low uptake velocity and that the 2.6 % mass increase are not close to the true final value. This is presumably due to the massive macroscopic change of the formulation as it looked like one singular large particle after twelve weeks. An increased particle size results in a drastically decreased specific surface area of the formulation, which explains the vastly decreased uptake velocity. The 40/75 bulk sample showed a comparable uptake velocity as the samples that were stored at the milder conditions. This is probably because the bulk sample formed several droplets instead of a large singular particle. The droplets broke when they were withdrawn from the Eppendorf tube, which increased the surface area. The high reduction observed with the blister samples is unexpected. However, possible explanations are an incompletely sealed blister or high influences on the adsorption behaviour as a result from the heat sealing and resultant changes of the formulation.

Overall, every post-stability value was at least 30 % lower than the initial measurement. Therefore, it can be assumed that this formulation underwent significant physicochemical changes during storage. As seen with the basic formulation, this formulation did not show any events during DVS analyses.

XRPD analysis of the 40/75 samples show a very small peak at about  $19\ 2\theta$  (same position as seen in Figure 5–59, but a smaller peak). This indicates a possible recrystallisation of either AP or CBD.

DSC analyses of the post-stability samples of the SP formulations showed an interesting progression. The capsule sample of the AO formulation stored at 25 °C and 60 % rH (Figure 5–63) and the blister sample of the basic formulation stored in the refrigerator showed an irregularity at about 50 °C and a second one between 90 and 100 °C. However, no distinct

peaks are visible. The bulk and capsule samples of the basic formulation when stored at 40 °C and 75 % rH show a barely visible exaltation at about 64 °C, which is slightly lower than the measured melting point of CBD. However, this could be a sign of a beginning recrystallisation. The 40/75 bulk sample of the formulation with AP (Figure 5–64) shows a small peak at about 130 °C. This is above the literature melting point of AP of 114 – 118 °C [107], which is the component with the highest melting point. However, this could be a different crystal modification.

DSC Diagram, SP + CBD + AP, 25/60 Capsule Sample

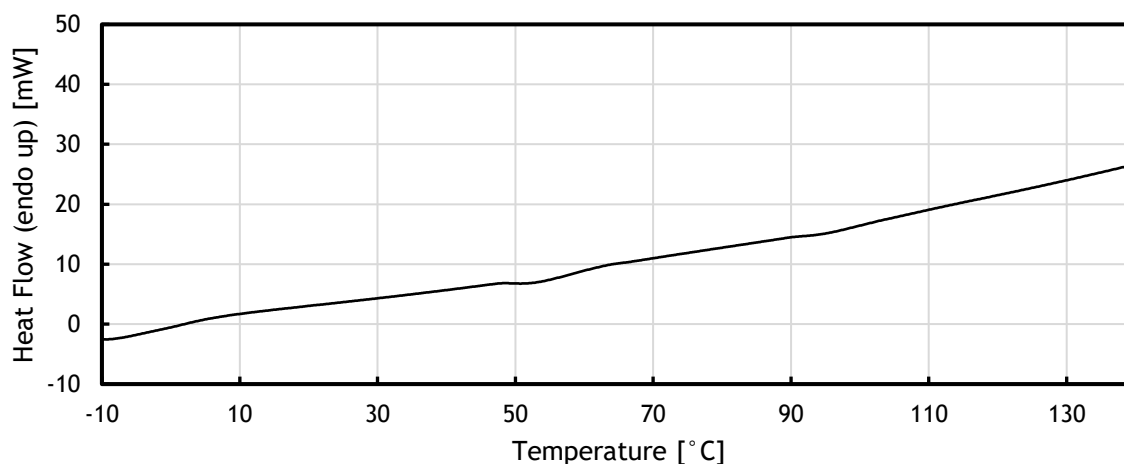


Figure 5–63: DSC diagram of the SP + CBD + AP formulation stored in a capsule at 25 °C and 60 % rH

DSC Diagram, SP + CBD + AP, 40/75 Bulk Sample

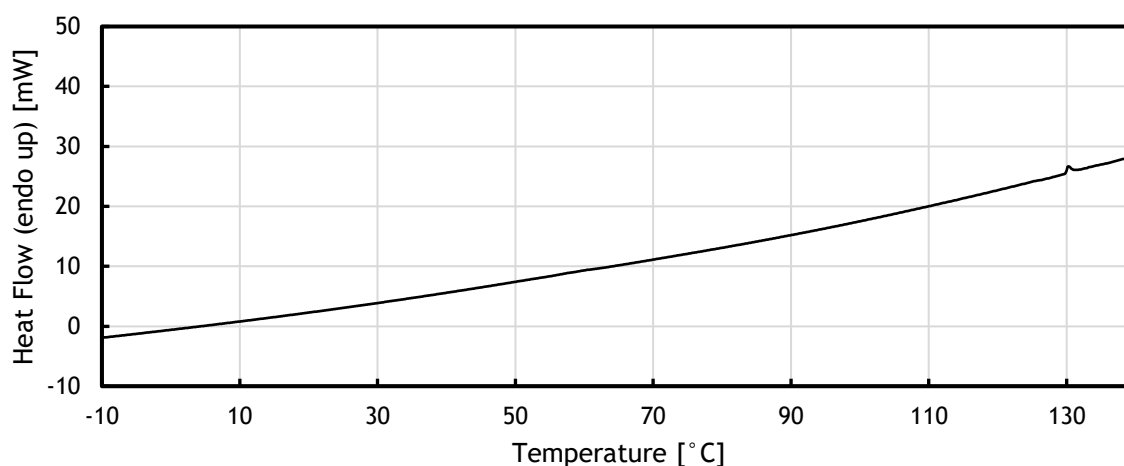


Figure 5–64: DSC diagram of the SP + CBD + AP formulation stored in bulk at 40 °C and 75 % rH

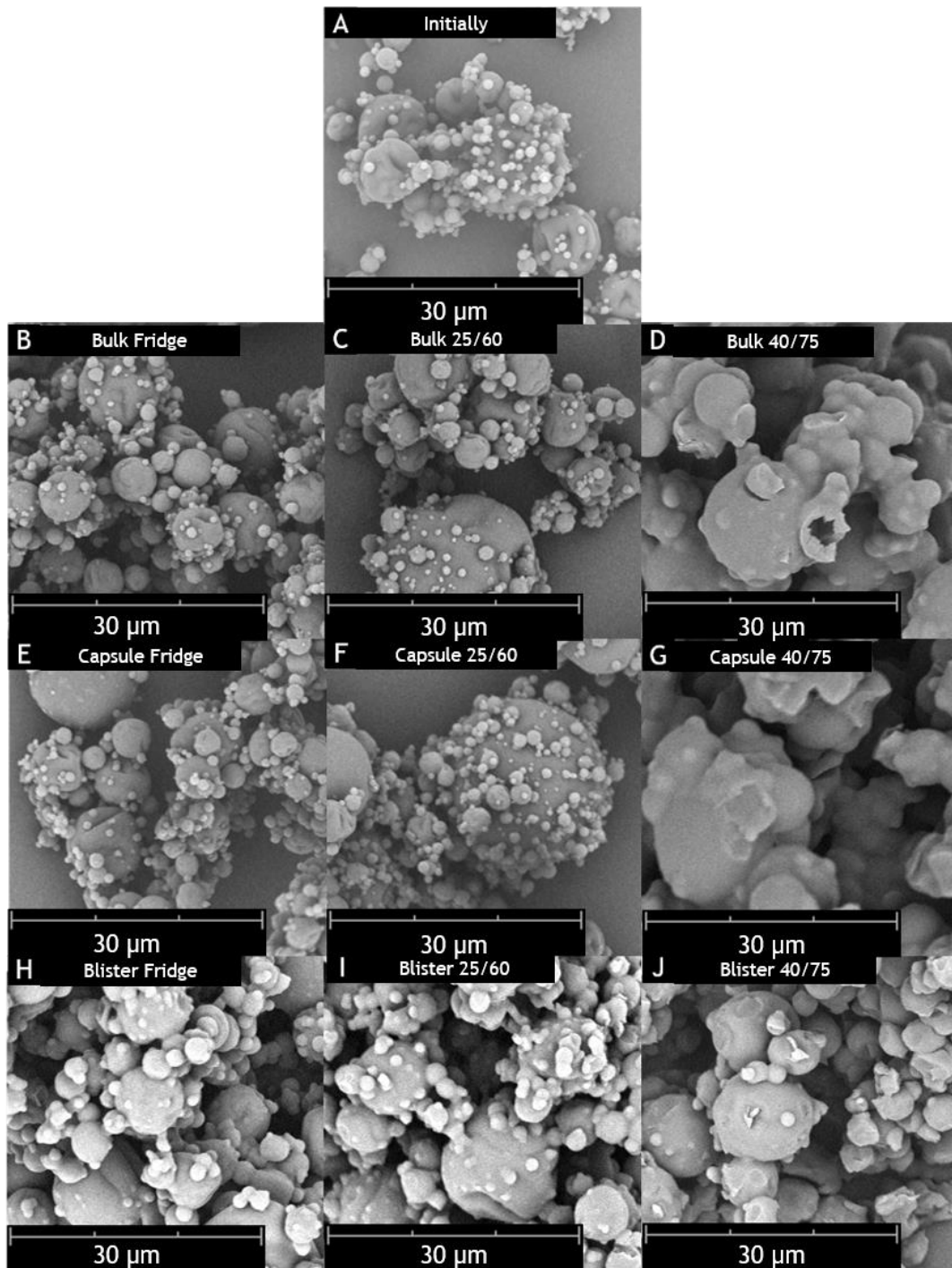


Figure 5—65: SEM images of the SP + CBD formulation, 2500x magnification; A - Initial; B-D: Bulk; E-G: Capsule; H-J: Blister; B, E, H: Fridge; C, F, I: 25 °C & 60 % rH; D, G, J: 40 °C & 75 % rH

Figure 5—65 shows SEM images of the SP basic formulation before and after stability. Storage in the refrigerator and at 25 °C and 60 % rH had no influence on the bulk and capsule samples (Figure 5—65 B, C, E, F). However, when the formulation was stored at 40 °C and 75 % rH the particles were fused together (Figure 5—65 D, G). Like the HPMC-AS formulation with AP, this is probably due to the high relative humidity. Storage in the blister also lead to fused particles (Figure 5—65 H-J). However, in this case, this is probably due to the sealing

of the blisters with 180 °C, which is clearly above the  $T_g$  of SP (see 3.1.2.3), as the milder storage conditions had no influence on the samples that were not packaged as securely.

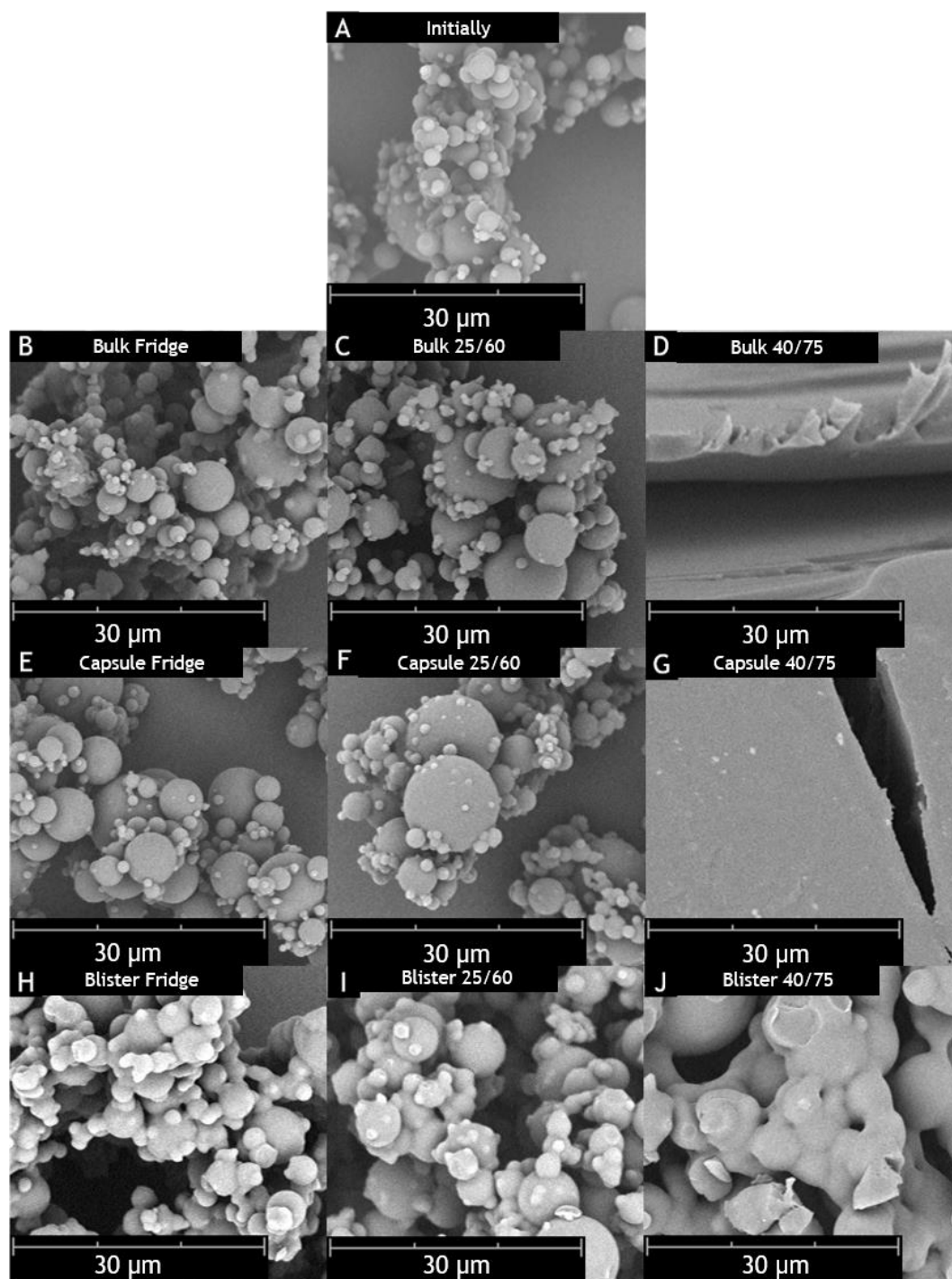
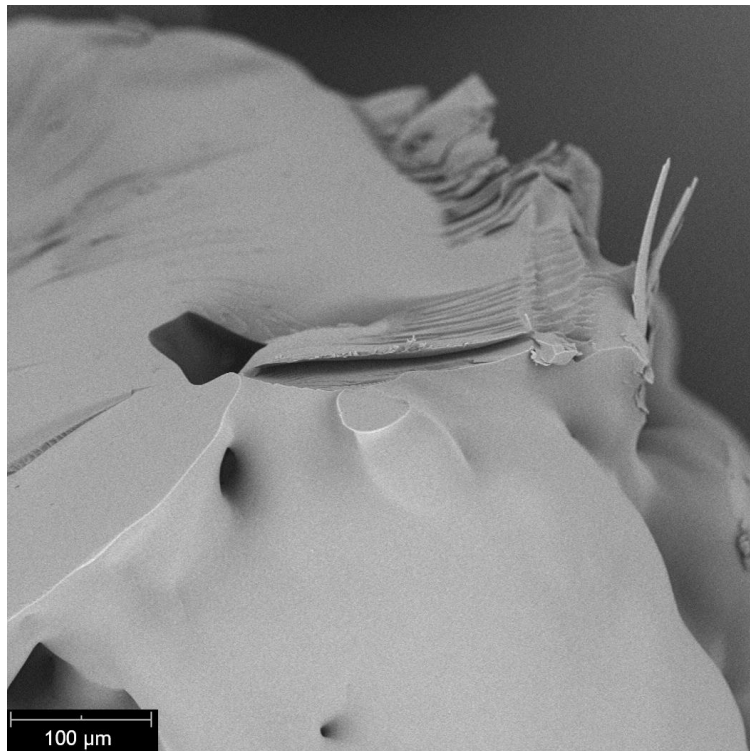


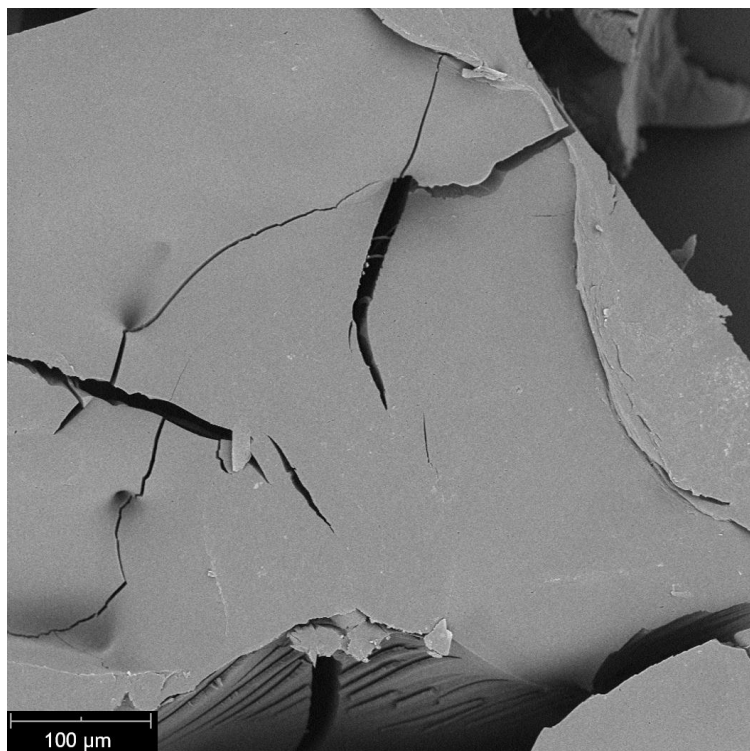
Figure 5—66: SEM images of the SP + CBD + AP formulation, 2500x magnification; A - Initial; B-D: Bulk; E-G: Capsule; H-J: Blister; B, E, H: Fridge; C, F, I: 25 °C & 60 % rH; D, G, J: 40 °C & 75 % rH

Figure 5—66 shows SEM pictures of the SP formulation with AP. Like the basic formulation, storage at the milder conditions had no influence on the particles (Figure 5—66 B, C, E, F), whereas the blister samples were fused together (Figure 5—66 H-J). However, storage at 40 °C and 75 % rH had by far the largest influence on this formulation. The bulk and capsule samples were not powdery anymore but one large particle (Figure 5—66 D, G). Therefore,

Figure 5—67 & 68, which show the 40/75 samples of the AP formulation at 500-fold magnification, were also added.



*Figure 5—67: 500x magnification of a SP + CBD + AP bulk sample, stored at 40 °C and 75 % rH*



*Figure 5—68 500x magnification of a SP + CBD + AP capsule sample, stored at 40 °C and 75 % rH*

## Twelve-Week Stability of the CBD Matrix Particles

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It has to be mentioned that the plugs had to be broken to be adhered to the used carbon sticker, this explains the breaking edge. These images confirm the assumption that the formulation lost any similarity with a powder and formed one large particle.

Data show that both formulations are very susceptible to the storage conditions. However, the formulation with AP showed more significant changes, especially after storage at 40 °C and 75 % rH. However, even storage in a blister, which should protect the formulation very efficiently, resulted in major changes. This leads to the conclusion that the basic formulation has to be favoured, although it shows obvious problems with the physicochemical stability itself.

#### 5.6.4 CHANGES OF THE AERODYNAMIC PERFORMANCE OF THE FORMULATIONS

The following chapters depict the differences between the aerodynamic performance of the formulations before and after the twelve-week stability study. Similar to previous chapters, three values in succession are in the order: refrigerated sample, 25/60 sample and 40/75 sample.

The analyses were carried out as described in chapters 4.2.7.1 and 4.2.7.2. One capsule was used for each DD run, whereas two capsules were used for the NGI runs. During the DD analyses, it became obvious that the macroscopic changes of the 40/75 samples of the HPMC-AS formulation with AP and both SP formulations prevented that the formulations could be dispersed successfully, as the formed agglomerates remained in the capsule after the inhalation manoeuvre. For this reason, these samples were analysed only once for their DD and excluded from NGI analyses. The experiments were carried out with the Cyclohaler<sup>®</sup> device and in a controlled environment (21 °C & 45 % rH).

Additionally, no analyses were performed with the Diskus<sup>®</sup> device, as initial experiments (see chapter 5.3.3) showed that the formulation could not be dispersed after being packaged in the blisters.

The recoveries are referenced to the amount of CBD that remained in the formulation after the stability study. This is calculated with equation (5–1). When the relative content was measured to be above 100 % the initial content was used.

$$m_{CBD\ post-stability} = m_{CBD\ initial} \cdot rel.\ content_{post-stability} \quad (5-1)$$

##### 5.6.4.1 HPMC Formulations

As seen with the initial characterisation, the recoveries of the DD analyses and thus the DDs itself are very variable. For the basic formulation, the recoveries were 43.2 ± 8.0 %, 70.6 ± 7.1 % and 77.8 ± 10.6 % (595.9 ± 112.4 µg, 917.9 ± 85.7 µg and 547.9 ± 77.4 µg) while the second formulation had recoveries of 73.3 ± 10.7 %, 59.4 ± 2.6 % and 58.8 ± 15.0 % (1020.6 ± 189.1 µg, 826.95 ± 39.2 µg and 830.1 ± 235.0 µg). The most probable reason for the low and variable recoveries is the utilisation of a less than optimal solvent for these analyses, due to the susceptibility of the inhalation device to acetonitrile (see chapter 5.3.3). However, it is visible that some recoveries were higher than the value of the respective initial measurement (basic: 66.7 %; AP: 62.6 %). This could be a result of a faster dissolution velocity, due to an increased water content (which was suspected as a reason for the changes in the water uptake, see 5.6.3.1). However, it would be expected that this would be the case for every sample of every formulation. This was not the case, as the 25/60 and 40/75 samples of the basic

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formulation had higher recoveries, whereas only the fridge sample of the AP formulation had an increased recovery.

Nonetheless, the calculation of the adjusted DD was considered essential for the comparison of the formulations before and after the stability study as well as the formulations with each other. The use of the adjusted DD eliminates the influence of the highly variable recoveries.

Table 5–22: Adjusted delivered doses of the HPMC formulations before and after the stability study (capsule) (left - basic formulation; right - AO formulation); n = 3

<i>HPMC + CBD</i>	<i>Mean adjusted DD (± SD) [%]</i>	<i>HPMC + CBD + AP</i>	<i>Mean adjusted DD (± SD) [%]</i>
<i>Initial</i>	94.7 (± 2.7)	Initial	90.2 (± 3.6)
<i>Refrigerated</i>	90.8 (± 0.5)	Refrigerated	95.7 (± 2.3)
<i>25 °C &amp; 60 % rH</i>	96.0 (± 1.6)	25 °C & 60 % rH	93.1 (± 1.5)
<i>40 °C &amp; 75 % rH</i>	92.0 (± 3.8)	40 °C & 75 % rH	95.1 (± 2.3)

Table 5–22 shows the mean adjusted DDs of both HPMC formulations. The differences of the post-stability values to the initial value is statistically insignificant ( $p > 0.087$ ). This indicates that emission from the capsule of these formulations was not influenced by the applied storage conditions, when the Cyclohaler® is used. When the post-stability samples are compared among one another, only the refrigerated and 25/60 samples of the basic formulation were different from each other ( $p = 0.006$ ). This could be a result of measuring inaccuracies for the other measurements and thus higher resulting SDs. Every other combination had a p-value  $> 0.174$ .

Table 5–23: Mean aerodynamic parameters of the HPMC + CBD formulation; n = 3

<i>HPMC + CBD</i>	<i>Mean FPD (± SD) [µg]</i>	<i>Mean FPF (± SD) [%]</i>	<i>Mean MMAD (± SD) [µm]</i>	<i>Mean Recovery (± SD) [%]</i>
<i>Initial</i>	283.2 (± 13.0)	24.6 (± 1.9)	3.9 (± 0.5)	79.4 (± 3.5)
<i>Refrigerated</i>	339.4 (± 20.4)*	24.8 (± 1.7)	3.6 (± 0.3)	89.8 (± 5.0)*
<i>25 °C &amp; 60 % rH</i>	262.2 (± 22.9)	23.4 (± 0.4)	3.7 (± 0.3)	89.8 (± 4.9)*
<i>40 °C &amp; 75 % rH</i>	147.8 (± 5.5)***	19.4 (± 1.9)*	4.7 (± 0.5)	107.4 (± 14.3)*

Table 5–23 shows the mean aerodynamic parameters of the HPMC basic formulation. Each post stability sample had a higher ( $p < 0.043$ ) recovery. Due to the differences in the recovery (which were higher after the stability study - probably due to an increased water content)



and the remaining CBD contents, the FPF is the most important parameter to compare the formulations before and after the stability study, as it explains why the increased FPD did not result in an increased FPF for the refrigerated samples. The 40/75 samples were the only ones which showed a significant ( $p = 0.029$ ) difference, in this case lower, FPF to the initial measurement.

When the post-stability samples are compared among one another, the FPDs are significantly different ( $p < 0.012$ ) for every pair (e.g. 25/60 vs 40/75). This could be a result of the unsteady recoveries or, especially for the refrigerated and 25/60 samples, a reduced CBD content in the formulation. A significant ( $p = 0.021$ ) difference for the FPFs was observable between the refrigerated and 40/75 samples. Additionally, the MMAD of the 40/75 samples was higher ( $p < 0.034$ ) than the value for the other post-stability samples, while there were no differences in the recovery.

These data show that storage in the refrigerator is the best option to maintain or even enhance the aerodynamic performance of this formulation. This could be due to a drop of electrostatic charges, which is often observable for formulations of this particle size [147]. However, it has to be kept in mind that the initial experiments were carried out about two weeks after the production of the formulations. This holding time could have already resulted in reduced electrostatic charges.

The influence of the storage on the CBD content was comparable to that of the aerodynamic behaviour as increasing stress had a more negative impact on the respective parameter(s).

Table 5—24: Mean aerodynamic parameters of the HPMC + CBD + AP formulation;  $n = 3$ ;  $n = 1$

HPMC + CBD + AP	Mean FPD ( $\pm$ SD) [ $\mu$ g]	Mean FPF ( $\pm$ SD) [%]	Mean MMAD ( $\pm$ SD) [ $\mu$ m]	Mean Recovery ( $\pm$ SD) [%]
<i>Initial</i>	251.0 ( $\pm$ 8.4)	22.1 ( $\pm$ 1.1)	4.6 ( $\pm$ 0.5)	81.1 ( $\pm$ 6.6)
<i>Refrigerated</i>	242.0 ( $\pm$ 19.0)	19.4 ( $\pm$ 1.5)	4.3 ( $\pm$ 0.4)	89.8 ( $\pm$ 0.4)
<i>25 °C &amp; 60 % rH</i>	283.1 ( $\pm$ 18.6)	24.4 ( $\pm$ 3.3)	4.9 ( $\pm$ 0.2)	82.1 ( $\pm$ 13.4)
<i>40 °C &amp; 75 % rH</i>	288.6 ( $\pm$ 71.0)	17.1 ( $\pm$ 4.6)	<u>5.3 (N/A)</u>	116.2 ( $\pm$ 19.2)*

Table 5—24 contains the mean aerodynamic parameters of the HPMC formulation with AP. The data of the 40/75 samples allowed only one MMAD calculation. This had been seen before with the initial measurements of the SP + CBD + AP formulation and is a result of the deposition profile of the formulation. The only significant difference between the initial data and the post stability data was the recovery of the 40/75 sample, which was higher ( $p = 0.040$ ).

Comparing the post-stability samples revealed a difference between the MMAD of the fridge samples and 25/60 samples. The refrigerated sample had a significantly lower value ( $p = 0.045$ ).

These data suggest that this formulation is relatively unsusceptible to the applied storage conditions, which was also the case for the CBD content of the capsule samples. However, the 25/60 samples seem to perform slightly better than initially. This is probably for the same reasons as with the refrigerated samples of the basic formulation, one of which could be the depression of electrostatic charges. Additionally, it must be mentioned that the recovery of the 40/75 samples was  $> 100\%$  which could have distorted the results.

Figure 5—69 shows the mean deposition profiles of the HPMC basic formulation before and after the stability study. Overall, the deposition before and after the stability study is reasonably consistent, as most of the formulation impacts in the throat and the preseparator, while the amount on the stages is reduced with every successive stage. The SDs of the throat and preseparator samples were smaller than before the stability study and, apart from the device of the 25/60 sample, generally relatively even. The high SDs for the preseparator and throat samples of the initial measurement could be a result of different agglomerate sizes in the individual capsules, as larger agglomerates are dispersed more easily. The capsules are not identical; therefore, the electrostatic charges might change in a different extent from capsule to capsule. This could also result in a different influence from the storage conditions. Both hypotheses could have also resulted in an uneven dispersion of the agglomerates. Table 5—10 (chapter 5.3.3) states that the particles which impact between stage two and six exhibit an inhalable size. Therefore, the deposition profile would ideally have a maximum at stage three or four. This signals that the formulation needs optimisation.

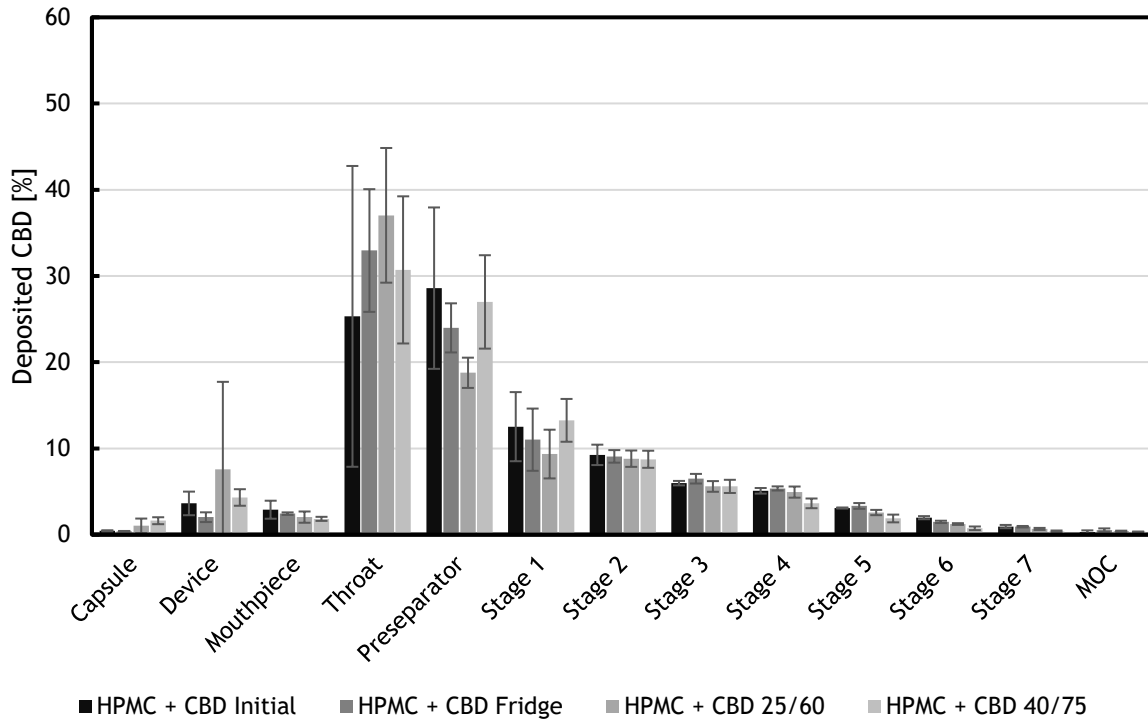


Figure 5–69: Mean deposition profile (NGI) of the HPMC + CBD formulation, initial and after the stability study;  $n = 3$ ; error bars = SD; Cyclohaler®

Figure 5–70 shows the mean deposition profiles of the HPMC formulation with AP before and after the stability study. There were only a few changes to the deposition behaviour after the stability study. Storage in the refrigerator resulted in a shift of the deposition from the initial stages to the throat, whereas the harsher storage conditions unexpectedly resulted in a shift from the throat to stage one. Every post stability sample had a lower retention in the device, while the trend of a lower deposition on every successive stage is consistent with the pre-stability results. Several samples (e.g. 25/60 throat) show high SDs, which could, be a result of different electrostatic charges or agglomerate sizes in the individual capsules, as well as the variable recoveries of the sample (see Table 5–24).

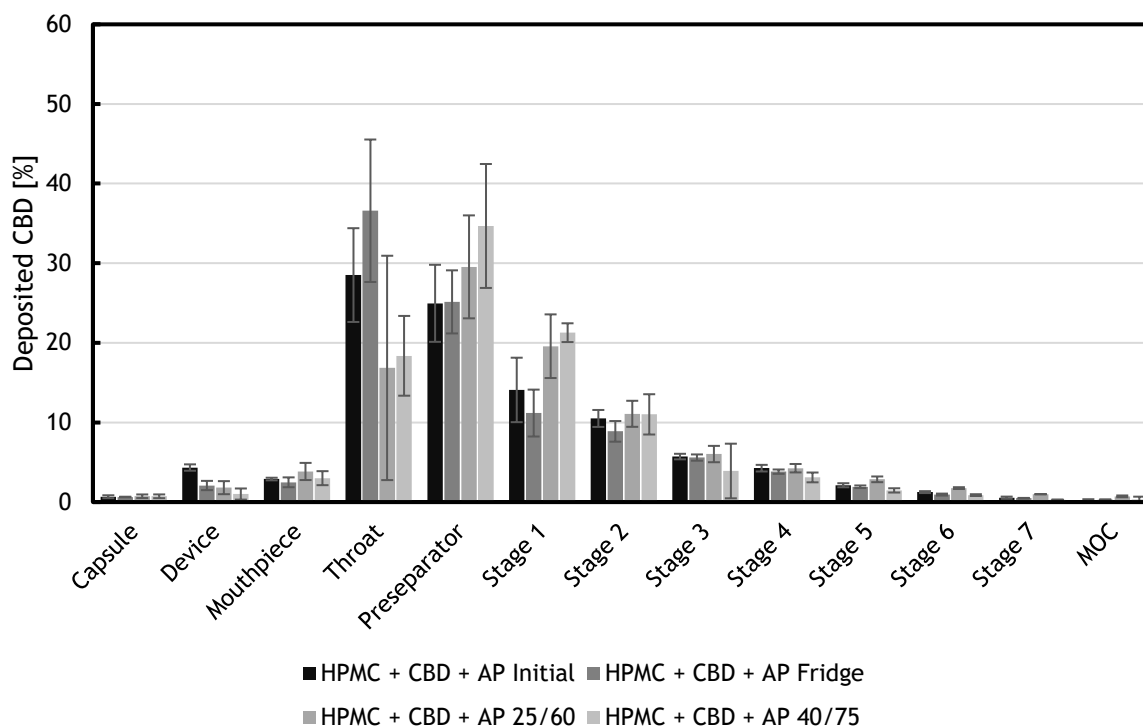


Figure 5–70: Mean deposition profile (NGI) of the HPMC + CBD + AP formulation, initial and after the stability study;  $n = 3$ ; error bars = SD; Cyclohaler®

Initially, it was shown that the basic formulation is probably slightly superior. Additionally, the basic formulation seems to have benefited slightly from storage at the milder conditions, while both formulations exhibit a decreased aerodynamic performance after storage at 40 °C and 75 % rH.

The aerodynamic characterisation of the blister samples was not possible due to the changes that were induced by the sealing process. Therefore, it can just be suspected that unchanged blister samples would perform very similar before and after the stability study. This would probably also translate to the capsules if these were further enclosed in a blister. Therefore, the basic formulation has to be favoured.

As seen before the stability study, most of the NGI runs (analysis of two capsules) were out of specification (recovery 75 – 125 % of the DD). For the basic formulation two runs of the 25/60 samples met the specification (109.5 %, 122.7 %), whereas only one run of the formulation with AP was inside the defined boundaries (118.9 %).

### 5.6.4.2 HPMC-AS Formulations

As seen with previous DD analyses during this work the recoveries were very variable which resulted in difficulty to compare the different samples. Additionally, it was not possible to disperse or dissolve the 40/75 sample of the AO formulation in a reasonable amount of time

(< 30 min on the orbital shaker). This is probably the result of the formation of a robust plug (see chapter 5.6.3.2). Therefore, only one capsule was analysed during DD analyses and these samples were not analysed in the NGI.

For HPMC-AS basic formulation the DD analyses had recoveries of  $69.0 \pm 11.5\%$ ,  $71.6 \pm 10.0\%$  and  $69.7 \pm 11.6\%$  whereas the AP formulation showed recoveries of  $100.6 \pm 17.5\%$ ,  $130.8 \pm 53.1\%$  and  $22.9\%$  ( $n = 1$ ). It is visible that the recoveries of the refrigerated and 25/60 post-stability samples of the AP formulation were distinctly higher than before the stability study ( $56.2\%$ ), whereas the recoveries of the basic formulation were comparable to the initial value ( $72.4\%$ ). Interestingly, this formulation was the only formulation that had a higher water uptake after the stability study. Again, the high variability of the recoveries is the reason for the decision to use the adjusted DD for the comparison of the formulations.

Table 5—25: Adjusted delivered doses of the HPMC-AS formulations before and after the stability study (left - basic formulation; right - AO formulation);  $n = 3$ ;  $n = 1$

<i>HPMC-AS + CBD</i>	<i>Mean adjusted DD (<math>\pm</math> SD) [%]</i>	<i>HPMC-AS + CBD + AP</i>	<i>Mean adjusted DD (<math>\pm</math> SD) [%]</i>
<i>Initial</i>	90.6 ( $\pm$ 4.3)	Initial	85.5 ( $\pm$ 2.2)
<i>Refrigerated</i>	95.6 ( $\pm$ 1.8)	Refrigerated	94.1 ( $\pm$ 2.2)**
<i>25 °C &amp; 60 % rH</i>	91.9 ( $\pm$ 2.7)	25 °C & 60 % rH	87.6 ( $\pm$ 5.0)
<i>40 °C &amp; 75 % rH</i>	90.6 ( $\pm$ 2.9)	40 °C & 75 % rH	61.9 (N/A)

Table 5—25 contains the mean adjusted DDs for both HPMC-AS formulations. For the basic formulation, no differences ( $p > 0.135$ ) were observable. The formulation with AP, however, had a significantly ( $p = 0.009$ ) higher adjusted DD after storage in the refrigerator, which could be a result of a reduced of electrostatic charges. However, as the initial measurements were carried out about two weeks after the production of the formulations, the electrostatic charges should have been reduced by a large margin already. The plug of the 40/75 sample did not dissolve completely over the course of 30 minutes on the orbital shaker. Therefore, the sample was taken before the plug was completely dissolved, which is the reason for the relatively high adjusted DD for this sample. The delivered part of the formulation must be from powder adhered to the inner surface of the capsule, which was not involved in the formation of the plug and therefore dissolved rapidly. When the post-stability adjusted DDs were compared with each other, no significant difference was observable ( $p > 0.064$ ). As most of the samples showed no differences in the adjusted DDs it can be assumed that the storage conditions have very limited effects on the expulsion from the capsules, when the inhalation manoeuvre is performed with the Cyclohaler® device.

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Table 5—26: Mean aerodynamic parameters of the HPMC-AS + CBD formulation; n = 3

HPMC-AS + CBD	Mean FPD (± SD) [µg]	Mean FPF (± SD) [%]	Mean MMAD (± SD) [µm]	Mean Recovery (± SD) [%]
<i>Initial</i>	400.4 (± 25.7)	29.4 (± 1.6)	3.5 (± 0.3)	93.7 (± 0.8)
<i>Refrigerated</i>	298.5 (± 7.6)**	24.3 (± 1.3)*	2.9 (± 0.1)*	93.7 (± 2.3)
<i>25 °C &amp; 60 % rH</i>	293.0 (± 25.2)**	25.6 (± 5.0)	3.6 (± 0.2)	106.2 (± 11.7)
<i>40 °C &amp; 75 % rH</i>	373.3 (± 31.3)	32.0 (± 3.0)	3.7 (± 0.3)	127.8 (± 12.5)*

Table 5—26 shows the mean aerodynamic parameters of the basic HPMC-AS formulation. It is striking that the NGI analyses showed distinctly higher recoveries than the DD analyses. This could, as stated before, be a result of the increased volume of solvent that is used for these analyses. Unexpectedly, every parameter aside from the recovery of the refrigerated sample is lower than the corresponding value of the initial measurement ( $p < 0.022$ ). This is interesting, as a lower FPF often results in a higher MMAD due to shift in the deposition to the earlier stages. The only other differing post stability result was identified to be the FPD of the 25/60 sample, which was lower than initially ( $p = 0.007$ ). It must be noted that the recovery of the 40/75 samples was significantly higher ( $p = 0.042$ ) than initially. The value is distinctly larger than 100 %, which indicates that the degradation in the capsules is not uniform. This could be a result of differences of the individual capsules.

Comparison of the post-stability samples showed ( $p < 0.021$ ) differences for the FPD, FPF and MMAD of the fridge and 40/75 samples, which could, again, be a result of the distinctly higher recovery of the 40/75 samples. Additionally, the MMAD of the fridge samples was also lower than the value of the 25/60 samples ( $p = 0.010$ ), while the FPD of the 40/75 samples was higher ( $p = 0.026$ ) than that of the 25/60 samples.

Interestingly, data suggest that storage at the most stressful conditions had less impact on the aerodynamic behaviour than storage at the milder conditions. In contrast, the increasing storage conditions (stress) had an increasingly negative impact on the CBD content. However, it has to be kept in mind that the recovery of the 40/75 samples was above 100 % which could have distorted the results of the aerodynamic characterisation.

Table 5—27: Mean aerodynamic parameters of the HPMC-AS + CBD + AP formulation; n = 3

HPMC-AS + CBD + AP	Mean FPD (± SD) [µg]	Mean FPF (± SD) [%]	Mean MMAD (± SD) [µm]	Mean Recovery (± SD) [%]
<i>Initial</i>	288.0 (± 13.6)	27.9 (± 1.1)	3.7 (± 0.2)	75.5 (± 2.1)
<i>Refrigerated</i>	231.7 (± 17.4)*	29.1 (± 2.2)	3.5 (± 0.2)	88.4 (± 1.1)***
<i>25 °C &amp; 60 % rH</i>	173.5 (± 22.2)**	29.6 (± 2.8)	4.3 (± 0.2)*	93.7 (± 2.8)***

Table 5—27 contains the mean aerodynamic parameters of the HPMC-AS formulation with AP. Both post-stability samples have significantly higher recoveries ( $p < 0.001$ ) and lower FPDs ( $p < 0.012$ ). The FPF, however, was larger ( $p > 0.369$ ) after both storage conditions, which was probably a result of the decreased CBD content. Additionally, the MMAD of the 25/60 samples was identified to be higher ( $p = 0.030$ ) than before the stability study, which suggests a deposition on the earlier stages.

The comparison of the post-stability samples showed significant ( $p < 0.037$ ) differences for every parameter but the FPF. Here, especially the MMAD is important, as it signals that the formulation reaches deeper parts of the lung after being stored in the refrigerator, compared to being stored at 25 °C and 60 % rH. It can also be supposed that there is a trend showing that storage at harsher conditions is increasingly bad for the formulation. This can also be seen at the 40/75 samples, which could not be sufficiently emitted from the capsule.

However, as the FPD is dependent on the recovery and the remaining API content, this again exemplifies the importance of the FPF. These results suggest that this formulation's aerodynamic behaviour is not substantially influenced by the applied storage conditions.

Like the CBD content, the aerodynamic performance of the 25/60 samples was influenced more than that of the refrigerated samples. However, the CBD content of the 25/60 samples was decreased more than that of the 40/75 samples, which was inverted for the aerodynamic performance as the latter were not dispersible.

Figure 5—71 shows the mean deposition profiles of the HPMC-AS basic formulation before and after the stability study. Storage in the refrigerator results in a shift of the deposition from the preseparator and stage 1 to the throat, which suggests that storage in the refrigerator negatively impacts the aerodynamic performance of this formulation. This suggestion is confirmed by the aerodynamic parameters.

The samples, which were stored under harsher conditions, show a similar deposition profile to the initial measurement. However, the 40/75 samples have a slight shift from the preseparator to the stages, which indicates an increased aerodynamic performance due to these

storage conditions. This is unexpected, as a continued exposure to high humidity often leads to agglomeration or even agglutination. Although this cannot be confirmed statistically, the aerodynamic parameters are consistent with this assumption.

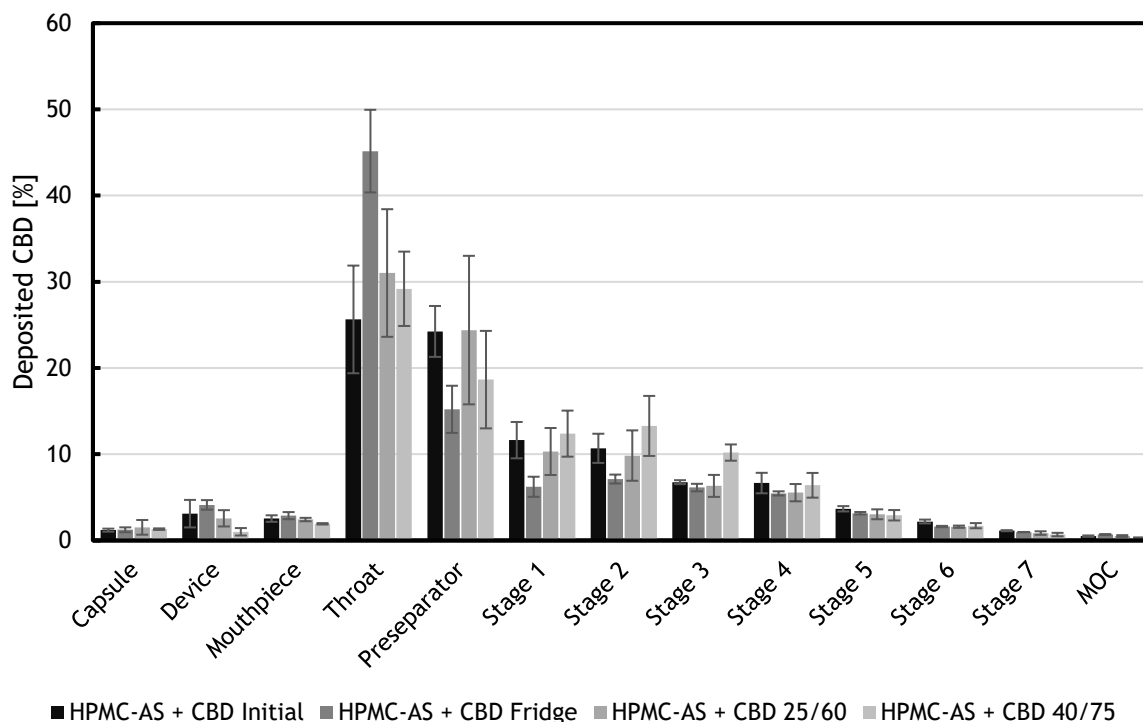


Figure 5–71: Mean deposition profile (NGI) of the HPMC-AS + CBD formulation, initial and after the stability study;  $n = 3$ ; error bars = SD; Cyclohaler®

Figure 5–72 shows the mean deposition profiles of the HPMC-AS formulation with AP. Storage in the refrigerator seems to result in slightly a marginal shift from stage 1 to stages 2 to 4 (lower MMAD, see Table 5–27). The 25/60 samples show in a shift from the throat to the preseparator as well as stages 1 and 2 (higher MMAD, see Table 5–27). However, the trend of a reduced deposition with successive stages is still observable. This formulation had comparatively low and consistent SDs overall. These deposition profiles indicate a slight improvement of the aerodynamic performance. Similar to the basic formulation this cannot be confirmed statistically but the FPF values seem to acknowledge this.



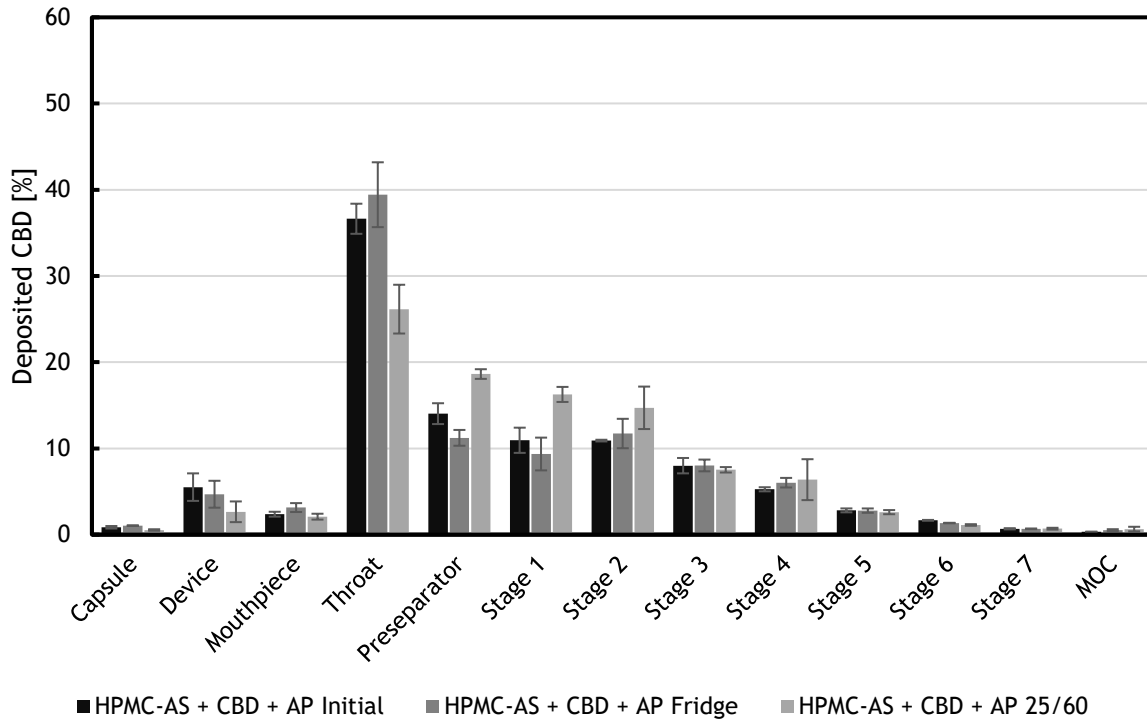


Figure 5–72: Mean deposition profile (NGI) of the HPMC-AS + CBD + AP formulation, initial and after the stability study;  $n = 3$ ; error bars = SD; Cyclohaler®

When both formulations were compared initially, the basic formulation was slightly superior to the AP formulation. However, the basic formulation seems to be more susceptible to the milder storage conditions, as the AP formulation performed slightly better after storage in the refrigerator than initially, whereas the basic formulation regressed in the FPF. Interestingly, the MMAD of both formulations was lower, which is an indicator for a deposition in deeper lung departments.

In contrast, storage at 40 °C and 75 % rH seemingly enhances the performance of the basic formulation. However, the recovery of 127.8 % could be an indication for distorted results for these samples. The formulation with AP is altered so significantly at 40 °C and 75 % rH that dispersion was practically impossible. It has to be considered that the capsules were not packaged further and analysis of the blister samples was not possible, as stated in chapter 5.3.3. However, it has to be expected that the blister provides excellent protection from the environment, which leads to the conclusion that the basic formulation is superior.

None of the samples of the basic formulation met the specifications (recovery 75 – 125 % of the DD). Interestingly each sample of the second formulation was inside the boundaries (fridge: 86.7 – 89.2 %; 25/60: 76.2 – 81.4 %).

5.6.4.3 SP Formulations

Similar to the HPMC-AS + CBD + AP formulation, these formulations underwent distinctive macroscopic changes during storage at 40 °C and 75 % rH (see chapter 5.6.3.3). The formed plugs were neither dispersible nor dissolvable in a reasonable timeframe and therefore only analysed once during DD analyses and excluded from NGI analyses.

The SP formulations had recoveries of 76.3 ± 9.2 %, 69.0 ± 16.2 % and 32.5 % (n = 1) (basic formulation) and 62.1 ± 23.4 %, 75.2 ± 7.2 % and 7.4 % (n = 1) (AP Formulation). For both formulations the recoveries were lower than before the stability study (basic: 79.3 %; AP: 80.5 %). However, as the recoveries generally showed very high SDs, they are relatively comparable. The high variability however reasons the decision to calculate the adjusted DD for the comparison of the formulations.

Table 5–28: Adjusted delivered doses of the SP formulations before and after the stability study (left – basic formulation; right – AP formulation); n = 3; n = 1

SP + CBD	Mean adjusted DD (± SD) [%]	SP + CBD + AP	Mean adjusted DD (± SD) [%]
Initial	95.4 (± 0.3)	Initial	95.2 (± 2.4)
Refrigerated	94.3 (± 1.5)	Refrigerated	92.7 (± 3.5)
25 °C & 60 % rH	96.1 (± 1.6)	25 °C & 60 % rH	95.0 (± 1.3)
40 °C & 75 % rH	23.3 (N/A)	40 °C & 75 % rH	12.5 (N/A)

Table 5–28 shows the adjusted delivered doses of the SP formulations. None of the samples analysed in triplicate showed differences (p < 0.331) to the initial adjusted DD. There also were no differences (p > 0.216) between the post-stability samples. The DD results indicate that the applied storage conditions have no effect on the emission of the formulation from the capsule, when the Cyclohaler® device is used, as long as the formulation did not sustain macroscopic changes.

Table 5–29: Mean aerodynamic parameters of the SP + CBD formulation; n = 3

SP + CBD	Mean FPD (± SD) [µg]	Mean FPF (± SD) [%]	Mean MMAD (± SD) [µm]	Mean Recovery (± SD) [%]
Initial	225.7 (± 24.1)	17.4 (± 2.9)	4.5 (± 0.1)	88.7 (± 5.2)
Refrigerated	217.4 (± 11.0)	16.1 (± 1.4)	4.3 (± 0.3)	93.2 (± 3.1)
25 °C & 60 % rH	184.4 (± 35.6)	14.0 (± 2.9)	5.5 (± 0.4)*	91.1 (± 2.7)

Table 5—29 shows the mean aerodynamic parameters of the SP basic formulation. Data shows that storage at both evaluated conditions only had marginal effects on the formulation, as the only significant ( $p = 0.013$ ) difference can be observed between the MMADs of the initial and 25/60 samples. The MMAD is also the only parameter which statistically different ( $p = 0.012$ ) when the post-stability samples are compared.

Although the differences between the FPFs are insignificant ( $p > 0.229$ ), there is a trend suggesting that the aerodynamic performance gets worse during storage, especially at higher humidities. This assumption is also strengthened by the fact that storage at 40 °C and 75 % rH almost completely inhibits the emission of the formulation from the capsules, which was shown during DD analyses.

In contrast, the CBD content did follow a trend and was generally unsusceptible to the applied storage conditions.

Table 5—30: Mean aerodynamic parameters of the SP + CBD+AP formulation;  $n = 3$ ;  $n = 1$

<i>SP + CBD + AP</i>	<i>Mean FPD (± SD) [μg]</i>	<i>Mean FPF (± SD) [%]</i>	<i>Mean MMAD (± SD) [μm]</i>	<i>Mean Recovery (± SD) [%]</i>
<i>Initial</i>	98.7 (± 5.9)	8.9 (± 0.8)	N/A	79.3 (± 1.3)
<i>Refrigerated</i>	118.5 (± 7.8)*	9.4 (± 0.9)	5.7 (N/A)	94.0 (± 4.1)**
<i>25 °C &amp; 60 % rH</i>	39.2 (± 2.7)***	3.4 (± 0.2)**	N/A	93.9 (± 2.1)***

Table 5—30 contains the mean aerodynamic data of the SP + CBD + AP formulation. Both post-stability recoveries are higher than initially ( $p < 0.004$ ). The FPD for the refrigerated samples was increased ( $p = 0.025$ ). This could be a result of the distinctly higher recovery, as the FPFs are not statistically different ( $p = 0.498$ ). The 25/60 samples showed a depression in FPF and FPD ( $p < 0.008$ ) although the recovery was significantly higher than initially. When the post-stability samples were compared among one another it could be seen that the refrigerated samples performed vastly superior, as both the FPD ( $p < 0.001$ ) and FPF ( $p = 0.007$ ) are significantly higher.

The initial deposition profile of this formulation did not allow the evaluation of the MMAD and only one of the post-stability runs enabled calculation of the MMAD, which explains the missing SD for the value of the refrigerated samples. However, this suggests that storage in the refrigerator slightly enhances the performance, maybe due to a reduction in electrostatic charges. However, as stated in the preceding chapters, the charges could also have been reduced before the initial measurements due to the holding time between production and experiments.

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There is a trend that this formulation is susceptible to the storage conditions, as the 25/60 samples performed significantly worse than the refrigerated samples, while the macroscopic changes of the 40/75 samples prevented a robust aerodynamic characterisation. This is a distinct difference to the influence of the storage on the CBD content, which was practically unaltered for every applied storage condition.

Figure 5–73 shows the mean deposition profiles of the base SP formulation. The stability study had no larger influences on the deposition behaviour. The trend of a reduced deposition with every stage is still visible after the stability study. The 25/60 samples, however, seem to deposit on the earlier stages, as the descent seems to be steeper. Additionally, it stands out that some SDs were distinctly higher than before, especially the device and stage 1 of the 25/60 samples. The SDs for the refrigerated samples were mostly smaller or comparable to the initial measurement, which could be a result of more evenly distributed electrostatic charges. These observations are consistent with the aerodynamic parameters.

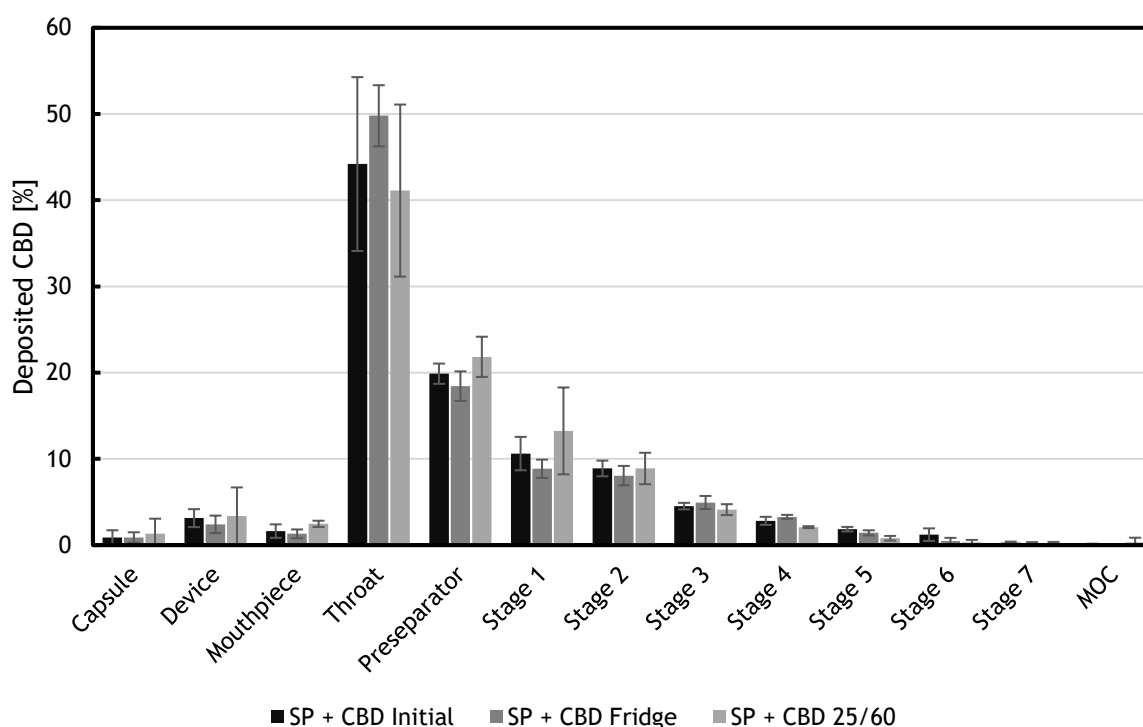


Figure 5–73: Mean deposition profile (NGI) of the SP + CBD formulation, initial and after the stability study;  $n = 3$ ; ; Cyclohaler<sup>®</sup> error bars = SD

Figure 5–74 depicts the mean NGI deposition profiles for the SP + CBD + AP formulation. Storage at 25 °C and 60 % rH resulted in a reduced deposition on the stages. In turn the deposition in the preseparator and especially the capsule was increased. The refrigerated samples showed a similar deposition to the initial measurement. There only was a shift from the

preseparator to the throat, which has no influence on the dose reaching the lungs. This formulation also showed relatively low SDs overall. The observations are consistent with the aerodynamic parameters of both formulations.

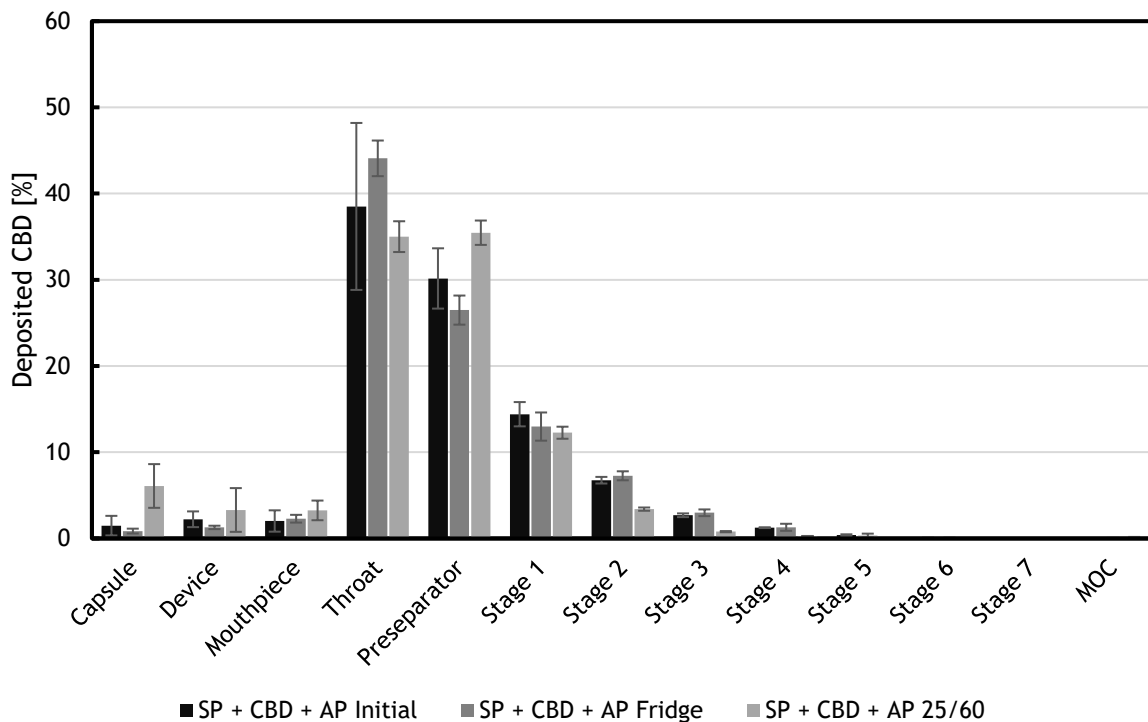


Figure 5—74: Mean deposition profile (NGI) of the SP + CBD + AP formulation, initial and after the stability study;  $n = 3$ ; error bars = SD; Cyclohaler®

Both formulations showed a poor aerodynamic behaviour. However, the basic formulation was clearly superior to the second formulation initially. This is also the case after the stability study, as it also seems to be less susceptible to the harsher storage conditions. Although the observed differences were insignificant, the AO formulation seemed to tolerate storage in the refrigerator more. Again, the blister samples were not evaluable, however it can be assumed that the blister would provide better protection from the environment than the capsule. Due to the overall very poor performance of the AP formulation, favouring of the basic formulation is mandatory.

One post-stability run of the basic formulation (fridge: 118.3 %) met the specification (recovery 75 - 125 % of the DD), whereas two 25/60 runs of the formulation with AP were inside the boundaries (117.9 %, 119.4 %).

## 5.7 DISCUSSION OF DPI FORMULATIONS

The following chapters compare the bulk properties, CBD protection and the aerodynamic behaviour of the different formulations. However, only the basic formulations will be compared among each other, as it was already seen that the basic formulation was at least slightly superior for every matrix.

### 5.7.1 CBD STABILITY

When the different matrices are compared for their post-stability CBD contents. It becomes obvious that SP has by far the best protecting capabilities of the three matrices. For each packaging and storage combination, the SP formulation had the highest remaining CBD content. The bulk samples stored at 40 °C and 75 % RH are the only sample set of this formulation that showed a remaining CBD content of < 96 %. Data also suggests that both the capsules and the blister can help in protecting the API from degradation.

Additionally, it can be seen that the HPMC formulation seems to tolerate the refrigerated and moderate storage better than the HPMC-AS formulation, whereas the harshest storage seems to be tolerated less. Interestingly, the capsule was sufficient to protect the CBD in the SP formulation from degradation. This was not the case for the other formulations.

Table 5–31: Post-stability (12 w) relative CBD contents of the basic formulations (top - bulk samples; middle - capsule samples; bottom - blister samples); n = 3; *n* = 2; *cursive*: n=1; \* SD

<i>Bulk samples</i>	<i>HPMC</i>	<i>HPMC-AS</i>	<i>SP</i>
<i>CBD [%], Fridge</i>	84.6 ± 13.7	75.9 ± 14.0	97.4 ± 3.8
<i>CBD [%], 25/60</i>	81.1 ± 0.3	<i>64.4 ± 7.7</i>	<i>96.7</i>
<i>CBD [%], 40/75</i>	49.4 ± 5.9	64.0 ± 1.9	82.9 ± 8.5
<i>Capsule samples</i>	<i>HPMC</i>	<i>HPMC-AS</i>	<i>SP</i>
<i>CBD [%], Fridge</i>	101.7 ± 0.7	<i>93.7 ± 0.1</i>	103.7 ± 2.6
<i>CBD [%], 25/60</i>	90.4 ± 6.5	76.1 ± 4.1	100.2 ± 0.6
<i>CBD [%], 40/75</i>	50.7 ± 3.6	62.6 ± 1.6	99.5 ± 4.6
<i>Blister samples</i>	<i>HPMC</i>	<i>HPMC-AS</i>	<i>SP</i>
<i>CBD [%], Fridge</i>	104.3 ± 0.6	<i>102.6</i>	113.1 ± 5.5
<i>CBD [%], 25/60</i>	106.6 ± 10.9	102.5 ± 2.0	108.1 ± 2.9
<i>CBD [%], 40/75</i>	98.7 ± 3.1	94.7 ± 11.1	120.0 ± 22.7

When the different matrices are compared for their post-stability CBD contents. It becomes obvious that SP has by far the best protecting capabilities of the three matrices. For each packaging and storage combination, the SP formulation had the highest remaining CBD content. The bulk samples stored at 40 °C and 75 % rH are the only sample set of this formulation that showed a remaining CBD content of < 96 %. Data also suggests that both the capsules and the blister can help in protecting the API from degradation.

Additionally, it can be seen that the HPMC formulation seems to tolerate the refrigerated and moderate storage better than the HPMC-AS formulation, whereas the harshest storage seems to be tolerated less. Interestingly, the capsule was sufficient to protect the CBD in the SP formulation from degradation. This was not the case for the other formulations.

Table 5—31 shows the relative post-stability CBD contents of the basic formulations.

Statistical analysis of the values of the HPMC and HPMC-AS formulations show differences between the three capsule samples ( $p < 0.033$ ) and the 40/75 bulk samples ( $p = 0.016$ ). For the 40/75 samples the HPMC-AS formulation had the higher relative CBD content, which is reversed for the other samples.

The same type of analyses for the HPMC and SP samples showed differences between the 40/75 bulk samples ( $p < 0.005$ ) and capsule samples ( $p = 0.000$ ). In both cases the SP matrix was superior in protecting the CBD.

The comparison of the HPMC-AS and SP samples revealed that the capsule samples ( $p < 0.022$ ) as well as the 40/75 bulk samples ( $p = 0.020$ ) were statistically different from each other.

Table 5—32 shows the relative post-UV CBD contents of the basic formulations. The progression is not comprehensible, especially as some values are higher than the corresponding post-stability values. However, it could again be observed that the SP formulation had the best CBD stability. The only sample where the SP formulation did not have the highest mean value was the refrigerated blister sample.

When the different matrices were compared with each other, the only difference between the HPMC and HPMC-AS formulations could be identified for the 40/75 capsule samples ( $p < 0.001$ ).

The comparison of the HPMC and SP formulations revealed that there were differences between the 40/75 samples ( $p < 0.001$ ; SP superior; no value for bulk samples ( $n = 1$  for HPMC)). Additionally, the remaining CBD content in the 25/60 capsule samples and the refrigerated bulk samples of the SP formulation was significantly higher ( $p < 0.038$ ).

## Discussion of DPI Formulations

Table 5–32: Post-UV relative CBD contents of the basic formulations (top - bulk samples; middle - capsule samples; bottom - blister samples); n = 3; cursive: n=1; ± SD

<i>Bulk samples</i>	<i>HPMC</i>	<i>HPMC-AS</i>	<i>SP</i>
<i>CBD [%], Fridge</i>	67.1 ± 11.4	75.0 ± 3.0	92.8 ± 9.2
<i>CBD [%], 25/60</i>	72.1 ± 4.7	65.3 ± 3.0	80.0 ± 5.6
<i>CBD [%], 40/75</i>	48.5	N/A	97.8 ± 3.1
<i>Capsule samples</i>	<i>HPMC</i>	<i>HPMC-AS</i>	<i>SP</i>
<i>CBD [%], Fridge</i>	93.1 ± 5.7	87.7 ± 7.6	95.9 ± 2.9
<i>CBD [%], 25/60</i>	84.5 ± 0.3	78.9 ± 5.7	95.2 ± 1.7
<i>CBD [%], 40/75</i>	45.9 ± 1.0	63.1 ± 1.5	89.5 ± 3.7
<i>Blister samples</i>	<i>HPMC</i>	<i>HPMC-AS</i>	<i>SP</i>
<i>CBD [%], Fridge</i>	120.0 ± 21.5	100.9 ± 3.4	104.4 ± 3.6
<i>CBD [%], 25/60</i>	104.4 ± 3.7	105.2 ± 4.8	122.7 ± 18.3
<i>CBD [%], 40/75</i>	93.4 ± 1.4	106.3 ± 21.1	107.8 ± 1.5

When the HPMC-AS and SP formulations were contrasted with each other, the bulk samples were identified to be different ( $p < 0.034$ ; SP superior; no value for 40/75 (n = 1 for HPMC-AS)) as well as the 25/60 and 40/75 capsule samples ( $p < 0.009$ ; SP superior).

The comparison of the remaining CBD content of the different basic formulations shows that SP is by far the best matrix when the stability of the CBD is the main focus.

### 5.7.2 PROPERTIES

Table 5–33 shows the initial properties of the basic formulations. It was seen that spray drying is an efficient way of production for these formulations, as every matrix showed adequate yields (> 65 %) while achieving excellent loading efficiencies (> 98 %).

When the densities of the formulations are compared, the values show a maximum difference of < 0.1 g/cm<sup>3</sup>, which is probably not relevant. These differences, however, are statistically significant ( $p < 0.001$ ), due to the high repeatability of the measuring method. After production, the HPMC-AS formulation had the lowest  $x_{50}$  ( $p < 0.019$ ) value but also a distinctly higher span value ( $p < 0.001$ ), which is probably a result of more stable agglomerates and thus a higher  $x_{90}$  value. The smaller particle size was expected, as it is the most lipophilic of the matrix substances and the formulations were spray-dried from an organic solvent, which results in a later precipitation during the procedure. This means that the shell is formed in a smaller



droplet and therefore cannot be as large as with the other polymers. The lipophilicity also explains that this formulation had the lowest water uptake.

As the formulations have densities above 1 g/cm<sup>3</sup>, a smaller particle size is very important to achieve a good aerodynamic performance. Therefore, it can be assumed that the HPMC-AS formulation will show a better aerodynamic behaviour than the other two produced formulations.

Table 5–33: Initial product characteristics of the basic formulations; *n* = 3; *n* = 10; *cursive*: *n* = 1; ± *SD*

	<i>HPMC</i>	<i>HPMC-AS</i>	<i>SP</i>
<i>Density [g/cm<sup>3</sup>]</i>	<u>1.2614 ± 0.0150</u>	<u>1.2309 ± 0.0090</u>	<u>1.1653 ± 0.0066</u>
<i>Particle Size (x<sub>50</sub>) [μm]</i>	6.84 ± 0.20	5.00 ± 0.27	5.65 ± 0.11
<i>Span</i>	2.73 ± 0.07	3.27 ± 0.05	2.27 ± 0.03
<i>Maximum Water Uptake [%]</i>	16.9	7.2	16.5
<i>Loading Efficiency [%]</i>	100.8 ± 0.1	98.6 ± 0.0	99.6 ± 0.1

The SP formulation is clearly more susceptible to stressful storage conditions than the other two formulations, which was unexpected as it has a comparable hygroscopicity to the HPMC formulation. This was obvious after evaluating the post stability 40/75 samples macroscopically, as the SP formulation formed a solid plug while the HPMC and HPMC-AS formulations retained the powdery state. The SP formulation also showed a more noticeable discolouration, which could be a sign of chemical changes, possibly of the API. However, SP has a relatively low *T<sub>g</sub>* of about 70 °C. If the *T<sub>g</sub>* of the formulation was lowered by incorporating CBD this could be a possible explanation for the changes. The *T<sub>g</sub>* of the finished formulation was not evaluated.

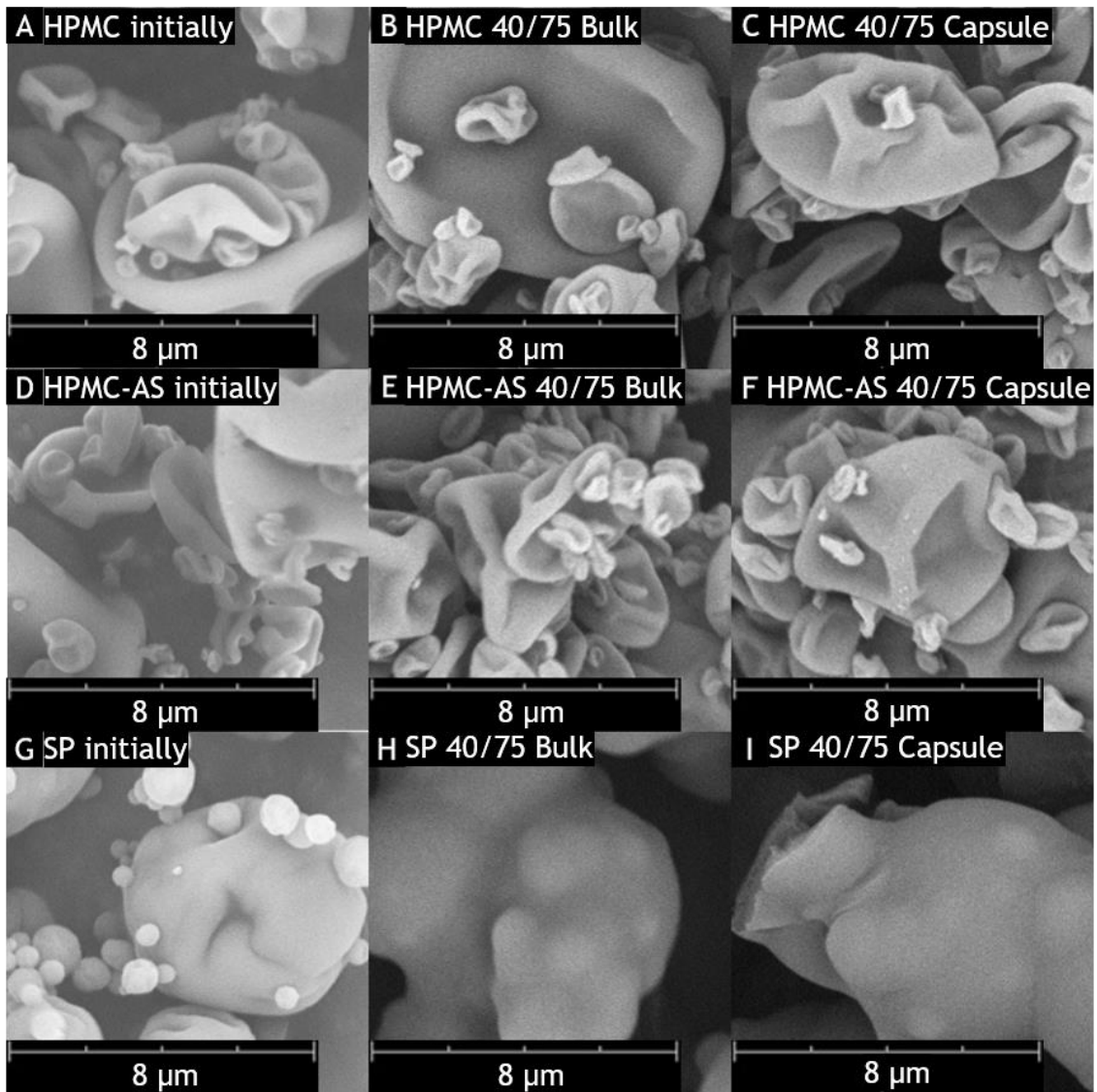


Figure 5—75: SEM images of the basic formulations; 10000x magnification; A-C HPMC; D-F HPMC-AS; G-I SP; A, D, G Initial; B, E, H 40-75 Bulk; C, F, I 40/75 Capsule

Figure 5—75 shows the three formulations before (A, D, G) and after storage at 40 °C and 75 % rH in bulk (B, E, H) or capsules (C, F, I). The images confirm that the SP formulation is more susceptible to the storage conditions than the other two formulations, as the particles have coalesced, probably due to the high humidity, whereas the other formulations look unaltered. After storage at 25 °C and 60 % rH, every formulation looked unaltered.

The formulations share the trait that the used blister sealing technique is not tolerated. This is probably due to the high temperature (180 C°) that is applied, as it results in the fusion of the particles and leads to an amalgamation of a portion of the formulation with the top foil.

Table 5—34 shows the post-stability  $x_{50}$  values of the formulations. Only the HPMC-AS formulation yielded three successful measurements for every sample. This is the first indication, that this formulation was influenced the least by the storage conditions.

After refrigerated storage, no difference ( $p > 0.159$ ) could be seen between the bulk samples of SP formulation and the other formulations, due to the high SD of the measurements of the SP formulation. The HPMC-AS formulation had a lower  $x_{50}$  ( $p < 0.029$ ) than the HPMC formulation after refrigerated storage and storage at 40 °C and 75 % rH.

Table 5–34: Post-stability  $x_{50}$  values of the basic formulations (top - bulk samples; bottom - capsule samples); cursive:  $n = 1$ ;  $\pm$  SD

<i>Bulk samples</i>	<i>HPMC</i>	<i>HPMC-AS</i>	<i>SP</i>
<i><math>x_{50}</math> [<math>\mu\text{m}</math>] Fridge</i>	6.02 $\pm$ 0.05	5.83 $\pm$ 0.09	6.58 $\pm$ 0.59
<i><math>x_{50}</math> [<math>\mu\text{m}</math>] 25/60</i>	7.47	6.39 $\pm$ 0.06	7.47
<i><math>x_{50}</math> [<math>\mu\text{m}</math>] 40/75</i>	7.90 $\pm$ 0.34	6.10 $\pm$ 0.16	N/A
<i>Capsule samples</i>	<i>HPMC</i>	<i>HPMC-AS</i>	<i>SP</i>
<i><math>x_{50}</math> [<math>\mu\text{m}</math>] Fridge</i>	5.83 $\pm$ 0.09	5.16 $\pm$ 0.37	6.58 $\pm$ 0.30
<i><math>x_{50}</math> [<math>\mu\text{m}</math>] 25/60</i>	7.70 $\pm$ 0.31	5.41 $\pm$ 0.5	6.38 $\pm$ 0.35
<i><math>x_{50}</math> [<math>\mu\text{m}</math>] 40/75</i>	8.25 $\pm$ 0.41	6.30 $\pm$ 0.44	N/A

Comparison of the capsule samples showed that the HPMC-AS formulation had a smaller mean particle size than the HPMC formulation at every storage condition ( $p < 0.039$ ) and than the SP formulation after refrigerated storage ( $p = 0.007$ ). Interestingly the differences between the HPMC formulation and the SP formulation were significant ( $p < 0.014$ ) after refrigeration or storage at 25 °C and 60 % rH. However, after refrigerated storage the SP formulation showed the higher  $x_{50}$ , while it was the other way around after storage at 25 °C and 60 % rH. These results could not be confirmed with the mean span values, as most of the values were not different from each other ( $p > 0.078$ ). The only difference ( $p = 0.048$ ) was seen between the refrigerated capsule samples of the SP ( $2.30 \pm 0.29$ ) and HPMC ( $2.82 \pm 0.13$ ) formulations.

The PSD results show that the HPMC-AS formulation is probably least susceptible to changes of the  $x_{50}$ . This was expected, as the higher lipophilicity of this matrix should result in less interplay of the water molecules with the particles. This results in less stable agglomerates

due to lower capillary forces and therefore an easier deagglomeration, which leads to a smaller  $x_{50}$  value.

Overall, these results show that the SP formulation is more susceptible to the storage conditions, especially the more stressful ones, than the other two formulations. It can also be seen that the HPMC-AS formulation is probably more resistant to the storage conditions. This is concluded from the fact that the storage did not result in difficulties to measure the  $x_{50}$  values. The changes between the initial values and the most stressed post-stability values is comparable. The lower  $x_{50}$  of the HPMC-AS samples was probably a result of the lower initial particle size.

### 5.7.3 AERODYNAMIC BEHAVIOUR

The initial aerodynamic characterisation (Table 5—35) of the three basic formulations showed that the HPMC-AS formulation has the best aerodynamic behaviour. This was concluded from the fact that both the FPD and FPF are significantly higher ( $p < 0.028$ ) than those of the other two formulations. Additionally, it had a higher recovery ( $p = 0.021$ ) than the HPMC formulation, which reduces the explanatory power of the FPD, and a lower ( $p = 0.004$ ) MMAD than the SP formulation. When the HPMC and SP formulation were compared, it is revealed that the HPMC formulation has higher FPD and FPF values ( $p < 0.024$ ). These observations confirm the expectation that the HPMC-AS formulation would perform best after production.

Table 5—35: Initial mean aerodynamic parameters of the basic formulations;  $n = 3$

	HPMC	HPMC-AS	SP
<i>FPD</i> [ $\mu\text{g}$ ]	283.2 $\pm$ 13.0	400.4 $\pm$ 25.7	225.7 $\pm$ 24.1
<i>FPF</i> [%]	24.6 $\pm$ 1.9	29.4 $\pm$ 1.6	17.4 $\pm$ 2.9
<i>MMAD</i> [ $\mu\text{m}$ ]	3.9 $\pm$ 0.5	3.5 $\pm$ 0.3	4.5 $\pm$ 0.1
<i>Recovery</i> [%]	79.4 $\pm$ 3.5	93.7 $\pm$ 0.8	88.7 $\pm$ 5.2

This can also be seen when examining the deposition profiles of the formulations (Figure 5—76), as the HPMC-AS formulation showed the highest deposition on the most relevant stages (two to six in this setting, see chapter 5.4.1). The SP formulation obviously had the worst performance, as it had by far the highest deposition in the throat, which subsequently leads to lower deposition on the stages.

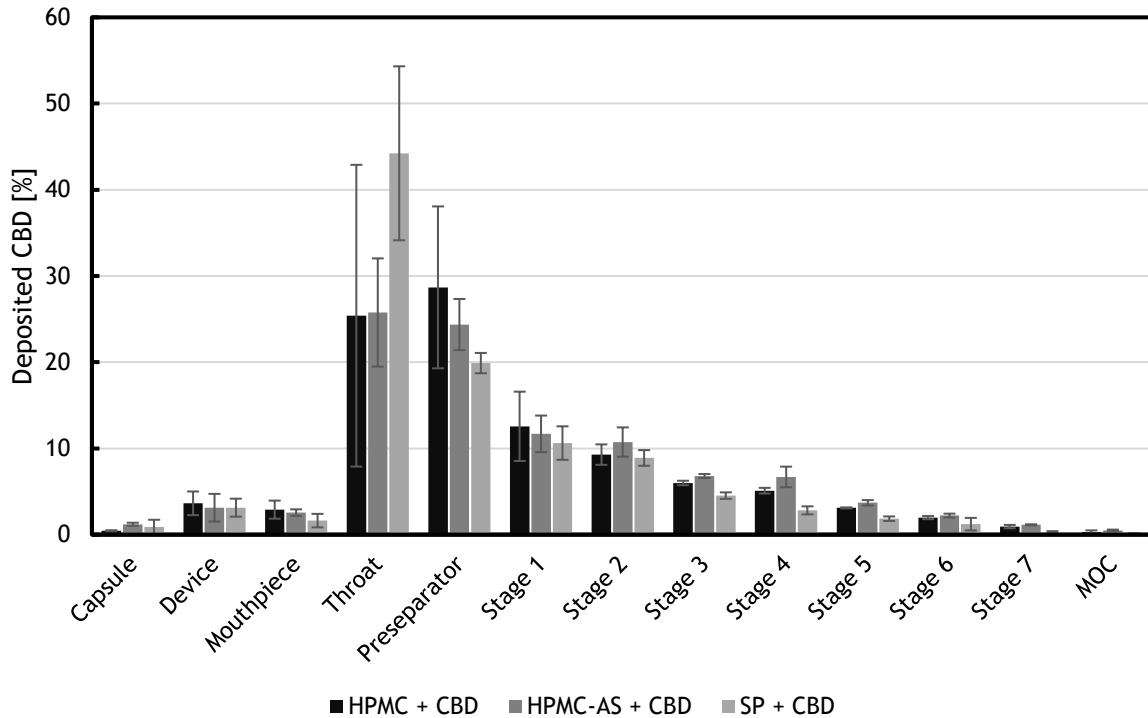


Figure 5—76: Initial mean deposition profiles (NGI) of the three basic formulations;  $n = 3$ ; error bars = SD; Cyclohaler®

Aerodynamic characterisation of the post-stability samples showed that the performance of the SP formulation was slightly decreased at each storage condition. However, the HPMC formulation showed a very slight (insignificant) increase of the performance after storage in the refrigerator, whereas the same could be seen for the HPMC-AS formulation when it was stored at 40 °C and 75 % rH, which was unexpected due to the reaction inducing conditions (Table 5—36). Fehler! Ungültiger Eigenverweis auf Textmarke. shows the mean aerodynamic parameters of the samples that were negatively impacted the least. When these were compared among one another, the HPMC and HPMC-AS formulations showed higher FPD and FPF values ( $p < 0.003$ ) than the SP formulation. The HPMC-AS formulation also had a higher FPF than the HPMC formulation ( $p = 0.023$ ). However, as the HPMC-AS samples had an exceedingly high recovery ( $> 100\%$ , significantly more than the other formulations ( $p < 0.010$ )), it could also be possible that the aerodynamic results are distorted. The deposition profiles (see respective chapters) show the superiority of the HPMC-AS formulation. It exhibited a higher deposition on stages two to six, whereas deposition in the throat and the preseparator was lower.

## Discussion of DPI Formulations

Table 5—36: Mean aerodynamic parameters of the basic formulations, after least (aerodynamically) impacting storage condition;  $n = 3$

	<i>HPMC, fridge</i>	<i>HPMC-AS, 40/75</i>	<i>SP, fridge</i>
<i>FPD [μg]</i>	339.4 ± 20.4	373.3 ± 31.3	217.4 ± 11.0
<i>FPF [%]</i>	24.8 ± 1.7	32.0 ± 3.0	16.1 ± 1.4
<i>MMAD [μm]</i>	3.6 ± 0.3	3.7 ± 0.3	4.3 ± 0.3
<i>Recovery [%]</i>	89.8 ± 5.0	127.8 ± 12.5	93.2 ± 3.1

Table 5—37 shows the aerodynamic parameters of the formulations after storage at the most negatively influencing conditions. For the HPMC formulation this was 40 °C and 75 % rH, while the other two formulations were influenced the most at 25 °C and 60 % rH. It must be remembered that no aerodynamic characterisation of the SP formulation after storage at 40 °C and 75 % rH was carried out due to the macroscopic changes, which inhibited a meaningful analysis.

Contrasting these values among each other showed that the HPMC-AS had higher FPD and lower MMAD values ( $p < 0.021$ ) than both other formulations, as well as a higher FPF than the SP formulation ( $p = 0.027$ ). There were no differences between the HPMC and SP formulations. These observations could also be made from the respective deposition profiles (see respective chapters), as the drop from stage to stage was lower for the HPMC-AS formulation, which resulted in the highest deposition on stages two to six of the three formulations.

Table 5—37: Mean aerodynamic parameters of the basic formulations, after most (aerodynamically) impacting storage condition;  $n = 3$

	<i>HPMC, 40/75</i>	<i>HPMC-AS, 25/60</i>	<i>SP, 25/60</i>
<i>FPD [μg]</i>	147.8 ± 5.5	293.0 ± 25.2	184.4 ± 35.6
<i>FPF [%]</i>	19.4 ± 1.9	25.6 ± 5.0	14.0 ± 2.9
<i>MMAD [μm]</i>	4.7 ± 0.5	3.6 ± 0.2	5.5 ± 0.4
<i>Recovery [%]</i>	107.4 ± 14.4	106.18 ± 11.7	91.1 ± 2.7

Due to the superiority of the HPMC-AS formulation after storage, it was decided to compare the most influenced samples of this formulation with the initial values of the other formulations. The statistical evaluation showed that the post-stability HPMC-AS samples had a lower MMAD ( $p = 0.004$ ) and a higher FPD ( $p = 0.029$ ) than the SP formulation initially. The FPF is not statistically different ( $p = 0.074$ ), due to the high SD of the HPMC-AS formulation, although

the mean value is distinctly higher (25.6 % vs. 17.4 %). In comparison with the initial aerodynamic results of the HPMC formulation, no significant differences could be identified. However, the FPD (293.0  $\mu\text{g}$  vs. 283.2  $\mu\text{g}$ ) and FPF (25.6 % vs. 24.6 %) mean values of the HPMC-AS formulation were higher. The mean deposition profiles of these formulations are depicted in Figure 5–77. Although, high SDs are visible, the deposition profiles suggest that the negatively influenced HPMC-AS formulation is slightly superior to the HPMC formulation. This is indicated by the slight shift from stage 1 to stages 2 – 4. The SP formulation, however, clearly appears inferior to the other formulations. This is mainly demonstrated by the very high deposition in the throat at the expense of a lower deposition on the stages.

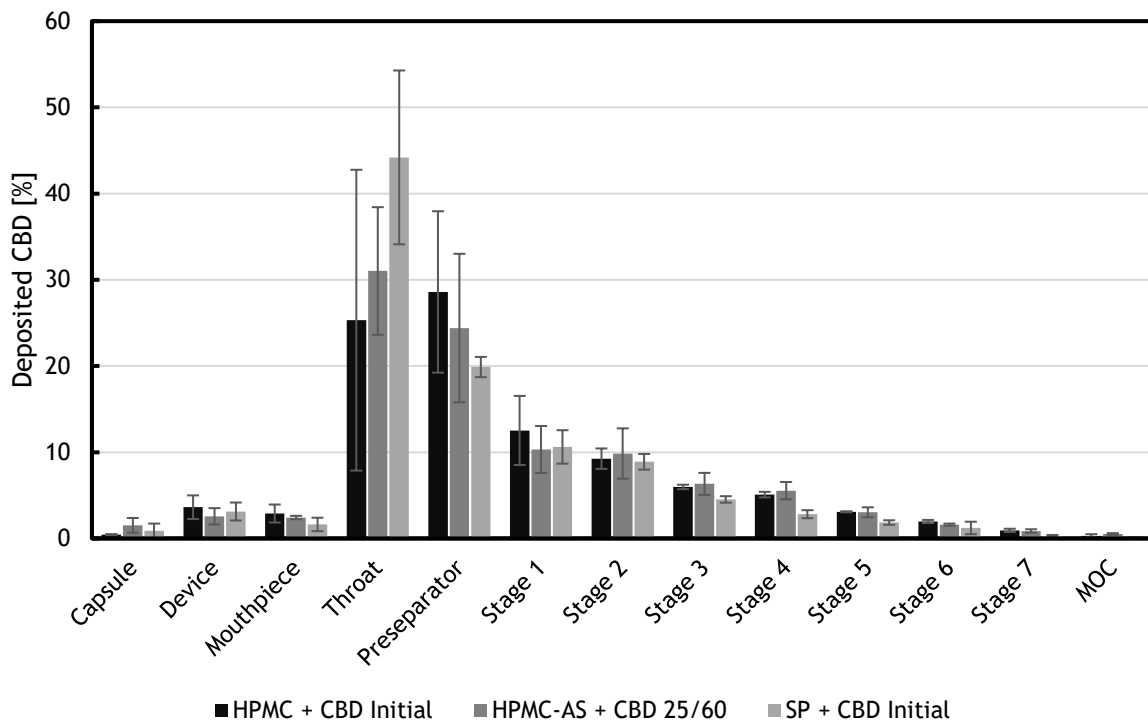


Figure 5–77: Mean deposition profiles (NGI) of the initial HPMC and SP basic formulations and the most influenced HPMC-AS basic formulation;  $n = 3$ ; error bars = SD; Cyclohaler®

Concluding it can be said that the HPMC-AS formulation shows the best aerodynamic behaviour of the different formulations, as the performance is comparable to that of the HPMC formulation initially and superior to that of the SP formulation initially, even after the most influencing storage.

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## 6 OVERALL FINDINGS, CONCLUSION & OUTLOOK

The first part of this work covered the production and examination of CBD pMDIs. During these experiments, it was found that the production of pMDIs is easily possible. However, it was also shown that these devices are not suitable for applying sufficient doses of the model drug (approximately 2.5 – 10 mg) to the lungs efficiently. This lack of efficiency would result in the need to apply two to seven puffs of the formulation, which was considered optimal, three times a day. Over the course of the formulation experiments the magnitude of the UV-induced degradation became clear. The best FPFs that were found during aerodynamic assessment of the generated formulations were 34.0 % and 35.1 %. Literature shows that solution pMDIs with HFA 134a can potentially achieve FPFs of up to 80 % and > 40 % when they contain 30 % EtOH. The FPF is inversely proportional to the orifice diameter [34], which matches the results of this study. If pMDIs were to be researched further, the main points of interest will have to be an enhancement of the aerodynamic performance, ideally coupled with increased API concentrations in the formulation. This could for example be achieved with smaller orifice diameters (performance) or different propellants or solubilisers (concentration). It also has to be investigated if the usage of a larger dosing valve (e.g. 100 µL available from Bespak Europe Ltd) has a negative influence on the aerodynamic performance, as this would be the easiest way to achieve higher doses.

Due to the visible degradation process, it was considered necessary to identify the main products of this process. For this task, HPLC-MS was implemented. These experiments, however, revealed that the photodegradation of CBD results in a multicomponent mixture without clear main degradants, which is in agreement with literature [142]. Therefore, a gradient method, which ensured that the CBD peak was clearly separated from the degradants, was developed and following analyses exclusively quantified the CBD content.

Additionally, the protective capabilities of four different antioxidants regarding CBD stability were evaluated. These experiments showed that ascorbic acid and its derivative ascorbyl palmitate are much more efficient than butylated hydroxyanisole or butylated hydroxytoluene in this task. Ascorbyl palmitate was considered more promising than ascorbic acid, as it is more lipophilic. The better matching lipophilicities should result in an easier formulation of solid dispersions, which were chosen as the formulation for a CBD containing dry powder. Preliminary experiments with solid dispersions showed that the API is not stable. This showed the need to find a way to stabilise the CBD.

In addition to UV stability, thermostability of the model drug was investigated. However, the results showed issues in the experimental build-up. The progression of the measured CBD concentrations was questionable, which was probably caused by evaporation and inaccurate



replenishment of solvent. Therefore, the results of this experiment can only be considered an indication for thermal instability of the model drug. The experiment was not redone, as the experimental build-up requires an extensive optimisation process.

Formulation trials showed that it is possible to efficiently prepare solid dispersions of CBD in the three selected matrices. The formulations showed mean geometric particle sizes near the upper threshold of the inhalable range (aerodynamic diameter 5  $\mu\text{m}$ ), which is a first indication for possible inhalability. Here, it has to be kept in mind that the aerodynamic and geometric diameter are not interchangeable. The density and the particle shape of particles have an influence on the aerodynamic diameter (see equation (2–1)). It was also possible to incorporate an equimolar (to the API) amount of the chosen antioxidant. This slightly altered the physicochemical properties and particle sizes of the formulation. These changes were expected due to the different percental amount of matrix in the formulation, which means that less matrix is available to incorporate twice the amount of molecules. Future research in this area should investigate if it is possible to further reduce the particle size of the formulations when CBD is incorporated.

The API content of the produced formulations, with and without the antioxidant, the unprocessed material, solutions as well as pMDIs was monitored over a twelve-week stability study. Unfortunately, unforeseeable issues with the analytical equipment occurred during the stability study. This required the exclusion of two scheduled content analyses (week two and four) as well as some individual sample runs at other time points (see chapter 5.6.2). The excluded runs, however, could not be repeated due to solvent evaporation of the already injected samples and the scheduling of the analyses.

Due to the analytical problems, some samples were not analysed in triplicate, which reduces the value of these results. Therefore, they have to be evaluated cautiously and should not be considered definite.

In summary, these results indicate that the CBD is most stable (chemically) when it is incorporated in the SP matrix. Additionally, the best storage condition for these formulations seems to be the refrigerator, which was expected as chemical reactions proceed at a lower rate at reduced temperatures. Interestingly, the addition of AP is not beneficial for the stability of the API. Even in comparison to the preliminary experiments the addition of AP seemed to reduce the stability in HPMC and HPMC-AS matrices. This was unexpected due to the positive effect observed in a solution and could be a result of the immobilisation of the molecules and possibly an accumulation of CBD near the surface of the particles. Regarding the packaging, the blister exhibited superior protection from chemical degradation to bulk and capsule samples. However, the formulations did not tolerate the sealing procedure, which shows that the

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physical stability of the formulations was not sufficient for this type of packaging procedure. It was not possible to differentiate between the capsule and bulk samples regarding the ability to physically stabilise the formulations. Future research in this area should investigate different blister-sealing techniques and blistered capsules. Additionally, different (higher) CBD contents in the formulations should be investigated, as for example Soluplus® has been shown to be able to incorporate up to 50 % of its own mass of substances [78], while it was possible to incorporate 25 % of CBD into the HPMC and HPMC-AS matrices. It would also be reasonable to research alternative antioxidants.

Analyses of the physicochemical parameters before and after the stability study showed negative influences of the additionally incorporated AP, especially at more stressful storage conditions. This is probably a result of the higher amount of molecules which have to be incorporated by the reduced amount of matrix. Overall, it became obvious that the SP formulations are much more susceptible to physical changes induced by the storage conditions than the other two matrices. The basic formulations of the other two matrices seem to be largely resistant to the tested storage conditions. However, there were some issues with the laser diffraction analyses of the post-stability samples of the HPMC formulation, which suggests that the HPMC-AS formulation is slightly superior. The capsule had only marginal protective effects on the physicochemical parameters. It is not possible to evaluate the protective effects of the blister in this regard, as the blistering of the formulation already altered the formulations, which impeded analyses. Prospective research should investigate if it is possible to make the SP formulation more resistant to the applied storage conditions, possibly in a blistered capsule. These observations also show the importance of different blister-sealing methods (e.g. UV-sealing [148]) for formulations like these.

Analyses showed that the aerodynamic behaviour of the SP formulations is relatively poor, whereas the HPMC-AS formulation is distinctly superior but not great in this regard. It has to be kept in mind that these formulations have not been optimised for their aerodynamic performance. The superiority of the HPMC-AS formulation could be a result of the lower particle size. Unfortunately, it was not possible to assess the performance of the formulations from the Diskus® device, as the sealing process of the blisters distinctly altered the formulations. The result of this alteration was that the formulations could not be expelled from the blister cups. Storage influenced every formulation negatively, which exemplifies the need of further packaging for the capsules. Overall, the highest achieved FPD was about 400 µg (HPMC-AS basic formulation), which would result in a high amount of inhalations (2 – 8, thrice daily) if the desired doses were to be reached and thus reduce patient adherence. Future work on the aerodynamic behaviour of these formulations should investigate higher filling weights and

alternative (automated) filling techniques for the capsules. Additionally, the formulations could potentially be optimised by adding different excipients to reduce cohesion (e.g. magnesium stearate, amino acids) or by forming controlled agglomerates (compare with soft pellets 2.2.3). The differing protection of blistered capsules would also be interesting regarding the aerodynamic behaviour. Different sealing methods have to be investigated to make blister-based devices applicable. However, it has to be kept in mind that the volume of the blister cups is normally restricted, which would require the formulation of higher concentrated formulations to achieve the necessary dosage while maintaining a reasonable amount of applications.

Finally, the comparison of the different formulation types (DPI and pMDI) shows that both developed formulations have comparable aerodynamic performances. The best DPI formulation (HPMC-AS) had an initial FPF of 29.4 % and of 32.0 % after storage, whereas the pMDI formulations achieved FPFs of 34.0 % (medium concentration) and 35.1 % (low concentration). When the DPI formulation is compared with literature values, it has to be kept in mind that the Cyclohaler<sup>®</sup> is normally used for interactive blends. Lobo et al. reported FPFs of > 60 % for the Cyclohaler<sup>®</sup>, when they dispersed 1 – 2 µm budesonide particles, which were generated with supercritical fluids [149]. In contrast, the TOBI<sup>®</sup> Podhaler<sup>®</sup> reaches an FPF of 65.2 % (14.7 mg per capsule) with its commercial spray dried formulation and 74.9 % with another spray-dried tobramycin formulation. The authors were also able to achieve an FPF > 80 % with the capsule-based RS01 inhalation device [150]. On one hand this shows that it is possible to achieve very high FPFs with spray dried formulations and on the other hand that the inhalation device is an important factor for the performance of these formulations and that they should be considered during formulation optimisation. Overall, this shows that the formulations that were developed during this project are equal in their performance and that both offer potential for optimisation regarding the aerodynamic performance. This was already seen with the preliminary HPMC formulation (25 % content, CBD extract), which showed a superior aerodynamic performance in comparison to every formulation of the stability study. In conclusion, the potential of a dry powder formulation has to be considered superior, as they offer the possibility of applying distinctly higher doses than pMDIs while also exhibiting a better chemical stability.

Overall, this work presents a possible approach to make CBD (or physicochemically similar substances) available for dry powder inhalation. However, no recommendation for the matrix can be made, as the differences between HPMC and HPMC-AS could be a result of different particle sizes. It was shown that reasonable amounts of antioxidants cannot increase the

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stability of CBD in a solid dispersion, although this ability was shown for solutions, which leads to the suggestion not to discard SP as a matrix, as it had been superior in protecting the CBD from degradation. As no optimisation was possible to this point, the generated formulations have to be seen as a starting point for prospective research. Promising FPFs of up to 30 % were reached with the unoptimised formulations. This suggests the potential that this type of formulation possesses and that further research is needed.

As this dissertation project solely focussed on polymeric solid dispersions, future research in this area should also investigate the formulation of lipid matrices (solid lipid particles). Solid lipid particles have been investigated for inhalation before [74,81] while newer research involves the encapsulation of CBD in lipids [92] or the formulation of other cannabinoids (THC) in nanostructured lipid carriers [151]. Consequently, it can be argued that there still are intriguing approaches in this context. These need to be investigated in future research to maximise the potential for CBD and similar substances in pharmaceuticals and especially the inhalation field.

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## 7 ABSTRACT

Today, more than 40 % of marketed active substances and up to 60 % new chemical entities (possibly future active substances) show high levels of lipophilicity [152]. One group of extensively researched, extremely lipophilic actives are cannabinoids, which also often exhibit UV-lability. Cannabinoids can therefore be regarded as substances that are difficult to formulate.

The historical way of cannabinoid application is inhalation. The inhalative route can be considered as advantageous for drug application, as it enables a rapid effect and circumvents the first pass effect, which can lead to higher bioavailabilities (in this case 31 % vs 16 % see chapter 3.1.1) and therefore lower required doses. However, the formulation of inhalable pharmaceuticals is often more complicated than oral formulations. This is a result of the low (aerodynamic) particle size (0.5 – 5 µm) that is required to deposit the API in the desired areas of the lungs. Some substances cannot be micronised easily, due to their physicochemical properties, and particles in this size range often exhibit a strong tendency to agglomerate, due to increased cohesion.

This project focussed on the identification of a possible approach to formulate an inhalative product containing cannabidiol (CBD) which might also be applicable for other cannabinoids or novel compounds with similar physicochemical properties. Due to stability issues, the formulation as a dry powder was favoured over solution-based formulations like metered dose inhalers. Nonetheless, a comparison between a pressurised metered dose inhaler and a dry powder inhaler was conducted to evaluate the potential of these formulations.

For the formulation of a dry powder, the production of a solid dispersion of CBD in a polymeric matrix was regarded promising. For this type of formulation three polymers were evaluated (hydroxypropyl methylcellulose (HPMC), hydroxypropyl methylcellulose acetate succinate (HPMC-AS) and Soluplus®). To generate particles in the desired size range, a spray-drying process was developed for each individual matrix. Due to the instability of CBD, each formulation was produced with and without ascorbyl palmitate (AP), which was identified as an efficacious antioxidant for CBD in solution. It was possible to achieve excellent loading efficiencies (96 – 101 %) and solid yields (59 – 75 %) with the developed spray-drying procedures. Every formulation had a mean particle size between 4 µm and 7 µm. The formulations were characterised aerodynamically and achieved fine particle fractions (FPF) between 8 % and 30 % with the Cyclohaler® device. Aerodynamic assessment with the blister-based Diskus® device was not possible, due to physicochemical changes during the sealing procedure of the blisters.

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Furthermore, a twelve-week stability study with three different storage conditions (5 °C, 25 °C & 60 % rH and 40 °C & 75 % rH) was conducted. Afterwards, the influence of the different conditions on the physicochemical properties, the CBD content and the aerodynamic behaviour was evaluated. The former two evaluations were also done on differently packaged (bulk, capsule, blister) samples of the formulations, while content analyses were also performed on the unprocessed material and pMDIs. Due to analytical issues, the content analyses need to be evaluated with care. However, as most of the samples retained the highest CBD content when stored in the refrigerator (same packaging) or in a blister (same storage), it seems that that the formulations are most stable when stored in the blister and under refrigeration. Regarding the stability of CBD, Soluplus® was the most effective matrix, while HPMC-AS showed the best physicochemical stability and aerodynamic behaviour. Additionally, it was revealed that the addition of AP is not beneficial for the aerodynamic behaviour, the physicochemical stability or the stability of the API when formulated as a dry powder matrix and could possibly exhibit a negative influence on some of these parameters.

## 8 ZUSAMMENFASSUNG

Mehr als 40 % der aktuell zugelassenen Wirkstoffe und bis zu 60 % der aktuell erforschten Wirkstoffe zeichnen sich durch eine hohe Lipophilie aus [152]. Cannabinoide sind eine umfangreich beforschte Gruppe von extrem lipophilen aktiven Substanzen und weisen zusätzlich häufig eine UV Instabilität auf. Cannabinoide können folglich als schwierig zu formulierende Substanzen angesehen werden.

Traditionell werden Cannabinoide dem Körper über die Inhalation zugeführt. Die inhalative Applikation kann aufgrund eines schnellen Wirkungseintritts und der Umgehung des First-Past-Effektes, was zu einer erhöhten Bioverfügbarkeit (in diesem Falle 31 % statt 16 %, siehe Kapitel 3.1.1) und damit geringeren benötigten Wirkstoffdosen führen kann, als vorteilhaft angesehen werden. Allerdings ist die Formulierung von inhalativen Arzneiformen oft komplizierter als die von oralen. Dies liegt an der geringen (aerodynamischen) Partikelgröße (0,5 – 5  $\mu\text{m}$ ), welche der Wirkstoff aufweisen muss, um sich in den gewünschten Lungenarealen abzusetzen. Einige Substanzen, unter anderem die Cannabinoide, können aufgrund ihrer physikochemischen Eigenschaften nur schwer mikronisiert werden. Außerdem zeigen Partikel in diesem Größenbereich, aufgrund von erhöhter Kohäsion, häufig eine starke Agglomerationsneigung.

Dieses Promotionsprojekt beschäftigte sich schwerpunktmäßig mit der Entwicklung eines Formulierungsansatzes für ein inhalatives Produkt mit Cannabidiol (CBD), der auch für andere Cannabinoide oder Wirkstoffe mit ähnlichen physikochemischen Eigenschaften angewendet werden könnte. Die Formulierung eines Trockenpulvers wurde einer lösungsbasierten Formulierung (z.B. Dosieraerosol) gegenüber bevorzugt, weil Stabilitätsprobleme des Wirkstoffs in Lösungen wahrscheinlicher sind. Um das Potential von beiden Formulierungstypen einschätzen zu können, wurden Dosieraerosole und Pulverinhalatoren trotzdem verglichen.

Für den angestrebten Formulierungsansatz wurden feste CBD-Dispersionen in einer Polymermatrix als vielversprechend angesehen. Für diesen Formulierungstyp wurden drei verschiedene Polymere (Hydroxypropyl Methylcellulose (HPMC), Hydroxypropyl Methylcellulose Acetat Succinat (HPMC-AS) und Soluplus<sup>®</sup>) verwendet. Um Partikel in der erforderlichen Größe herzustellen, wurden Sprühtrocknungsprozesse für jede Matrix entwickelt. Aufgrund der Instabilität von CBD wurde jede Formulierung jeweils einmal mit und einmal ohne Ascorbylpalmitat (AP) hergestellt. AP wurde im Vorfeld als wirksames Antioxidans für CBD identifiziert. Die entwickelten Sprühtrocknungsprozesse wiesen ausgezeichnete Beladungseffizienzen (96 – 101 %) und solide Produktionsausbeuten (59 – 75 %) auf. Jede der Formulierungen hatte eine mittlere Partikelgröße zwischen 4 und 7  $\mu\text{m}$ . Außerdem wurde jede Formulierung

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aerodynamisch charakterisiert (Feinpartikelfractionen (FPF) 8 – 30 %). Dafür wurde der Cyclohaler® verwendet. Aufgrund von physikochemischen Veränderungen durch den Versiegelungsprozess der Blister, war es nicht möglich eine aerodynamische Charakterisierung mit dem blisterbasierten Diskus® Inhalator durchzuführen.

Des Weiteren wurde eine dreimonatige Stabilitätsstudie unter drei verschiedenen Lagerungsbedingungen (5 °C, 25 °C & 60 % rH und 40 °C & 75 % rH) durchgeführt. Hierbei wurde der Einfluss der verschiedenen Lagerungsbedingungen auf die physikochemischen Eigenschaften, den CBD-Gehalt und das aerodynamische Verhalten untersucht. Die ersten beiden Untersuchungen wurden des Weiteren auch auf unterschiedlich verpackte Proben der Formulierungen (Bulk, Kapsel, Blister) angewendet, während die Gehaltsanalysen auch mit dem Basismaterial und Dosieraerosolen durchgeführt wurden. Aufgrund von analytischen Schwierigkeiten können die Ergebnisse der Gehaltsbestimmungen nicht als gesichert angesehen werden. Da die meisten der Proben nachdem sie im Kühlschrank (selbe Verpackung) und im Blister (selbe Lagerbedingung) im Vergleich den höchsten CBD Gehalt hatten, legen die Ergebnisse nahe, dass diese Formulierungen am stabilsten sind, wenn sie verblistert im Kühlschrank gelagert werden. Soluplus® war in Bezug auf die Stabilisierung des CBD die effektivste Matrix, während HPMC-AS die beste physikochemische Stabilität und das beste aerodynamische Verhalten gezeigt hat. Außerdem wurde festgestellt, dass das Hinzufügen von AP für die aerodynamischen Eigenschaften sowie die physikochemische Stabilität und die Stabilität des Wirkstoffs nicht von Vorteil oder sogar nachteilig ist.



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## ***10 APPENDIX***

### ***10.1 LIST OF ABBREVIATIONS***

5-HT	Serotonin
AA	Ascorbic Acid
ACI	Andersen Cascade Impactor
AO	Antioxidant
AP	Ascorbyl Palmitate
API	Active Pharmaceutical Ingredient
aq. bidest.	Bidistilled Water
BHA	Butylated Hydroxyanisole
BHT	Butylated Hydroxytoluene
CBD	Cannabidiol
CD	Cyclodextrin
CFC	Chlorofluorocarbon
CNS	Central nervous system
COPD	Chronic Obstructive Pulmonary Disease
DD	Delivered Dose
DoE	Design of Experiments
DPI	Dry Powder Inhaler
DSC	Differential Scanning Calorimetry
DUSA	Dosage uniformity sampling Appartus
DVS	Dynamic Vapour Sorption
EtOH	Ethanol
FDA	Food and Drug Administration
FSI	Fast Screening Impactor
GRAS	Generally Recognized As Safe
HFA	Hydrofluoroalkane
HPLC	High Performance Liquid Chromatography
HPMC	Hydroxypropyl Methylcellulose
HPMC-AS	Hydroxypropyl Methylcellulose Acetate Succinate
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
MeOH	Methanol
MMAD	Mass Mean Aerodynamic Diameter
MOC	Multiple Orifice Collector

## List of Abbreviations

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MS	Mass Spectroscopy
MSLI	Multi-Stage Liquid Impinger
N/A	Not applicable; not available; no answer; not analysable
NGI	Next Generation Impactor
NRF	Neues Rezeptur Formularium
PEG	Polyethylene glycol
Ph. Eur.	European Pharmacopoeia
pMDI	(pressurised) Metered Dose Inhaler
PSD	Particle Size Distribution
rH	relative Humidity
SCG	Snap Cap Glass Vessel
SCOGS	Selected Committee on Generally Recognized as Safe Substances
SEM	Scanning Electron Microscopy
SMI	Soft Mist Inhaler
SP	Soluplus®
T <sub>g</sub>	Glass Transition Temperature
THC	Δ <sup>9</sup> -Tetrahydrocannabinol
TRP	Transient receptor potential channel
UV	Ultraviolet
XRPD	X-Ray Powder Diffraction

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## 10.2 UNITS

%	Per cent, 10 <sup>-2</sup>
°	Degree(s)
°C	Degree(s) Celsius
μ*	Micro (followed by other unit), 10 <sup>-6</sup>
A	Ampere
bar	Bar (10 <sup>5</sup> pascal)
g	Gram(s)
h	Hour (3600 seconds)
k*	Kilo (followed by other unit), 10 <sup>3</sup>
L	Litre (10 <sup>-3</sup> cubic metres)
m	Metre(s)
m*	Milli (followed by other unit), 10 <sup>-3</sup>
m <sup>2</sup>	square metre(s)
m <sup>3</sup>	Cubic metre(s)
min	Minute (60 seconds)
n*	Nano (followed by other unit), 10 <sup>-9</sup>
Pa	Pascal (N/m <sup>2</sup> or kg/(m*s <sup>2</sup> ))
s	Second(s)
V	Volt(s)
wt%	Mass fraction, 10 <sup>-2</sup>

### 10.3 MATERIALS

#### 10.3.1 STABILITY STUDY MATERIALS

Ascorbylpalmitate	Obtained from Sigma-Aldrich (Munich, Germany) Batch: BCBS8843V; CAS: 137-66-6
Cannabidiol	Cannapure® PH Graciously gifted by Symrise AG (Holzminden, Germany) Batch 18R18786; declared content 100.5 %; CAS: 13956-29-1
HPMC	Methocel™ E5 Premium LV Kindly donated by Colorcon GmbH (Idstein, Germany) Batch: OL02012402; CAS: 9004-65-3
HPMC-AS	AquaSolve™; Type: MF Kindly donated by Ashland Industries (Düsseldorf, Germany) Batch: 60F-710001; CAS: 71138-97-1
Soluplus®	Kindly donated by BASF SE (Ludwigshafen, Germany) Batch: 84414368E0; CAS: 402932-23-4

#### 10.3.2 MATERIALS FOR PRELIMINARY EXPERIMENTS

The materials of the stability study were used for these experiments as well

Ascorbic acid	Obtained from Caelo GmbH (Hilden, Germany) Batch: 10101107; CAS: 50-81-7
Butylated hydroxy-anisole	Obtained from Sigma-Aldrich (Munich, Germany) Batch: SLBB0317V; CAS: 25013-16-5
Butylated hydroxy-toluene	Obtained from Caelo GmbH (Hilden, Germany) Batch: 12382303; CAS: 128-37-0
CBD extract	Kindly donated by AiFame GmbH (Wald-Schönengrund, Switzerland) Declared content: 94 %
HPMC-AS	AquaSolve™; Type: LF Kindly donated by Ashland Industries (Düsseldorf, Germany) Batch: 55F-710001; CAS: 71138-97-1
HFA 134a	Obtained from Solvay Fluor GmbH (Frankfurt, Germany)
HFA 227	Obtained from Daikin Refrigerants Europe GmbH (Frankfurt, Germany)



### 10.3.3 ANALYTICAL MATERIALS

These were used over the course of the whole work

Acetonitrile	Riedel-de- Haën® HPLC Grade Honeywell International Inc.(Morristown, NJ, USA) Obtained from Walter CMP (Kiel, Germany) CAS: 75-05-8
Bidistilled water	Freshly produced with in-house facility San-Asalo-Sohlberg Corp. Finn Aqua 75 (Helsinki, Finland)
Ethanol	Absolute 99.9 %, Baker™ ANALYZED, J.T. Baker (HPLC Grade), Avantor (Center Valley, PA, USA) Obtained from Walter CMP (Kiel, Germany) CAS: 64-17-5
Nitrogen	Used for DSC and DVS in 5.0 quality (99.999 %) Used for spray drying in technical quality (≥ 99.8 %) Obtained from Linde GmbH (Hamburg, Germany)

### 10.3.4 PACKAGING AND INHALATION DEVICES

Cyclohaler®	Obtained from PB Pharma GmbH (Meerbusch Germany)
Diskus®	Obtained from GSK GmbH & Co. KG (Munich, Germany)
Capsules	HPMC capsule, size 3 VCaps® Plus Capsugel (Morristown, NJ, USA)
Blister top foil	Patz 48613 /Paper 50/PET 12/ALU-S 20 dull – peel off – unprinted Constantia Patz Ges.m.b.H (Loipersbach, Austria) Graciously gifted by Harro Höfliger Verpackungsmaschinen GmbH (Allmersbach im Tal, Germany)
Blister bottom foil	Patz 497 /25-45-100 coldform – unprinted Constantia Patz Ges.m.b.H (Loipersbach, Austria) Graciously gifted by Harro Höfliger Verpackungsmaschinen GmbH (Allmersbach im Tal, Germany)
61 µL dosing valves	DF316/61 RCU CS20 AG Kindly provided by Aptar Pharma (Le Vaudreuil, France)
50 µL dosing valves	DF 30/50 RCU CS 20 Argent Kindly provided by Valois Pharm (Le Vaudreuil, France)

## Materials

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pMDI orifices	0.22 mm, 0.30 mm, 0.42 mm Acquired from bespak Ltd (King's Lynn, England, UK)
Glass can	15 mL; Obtained from SGD Kipfenberg GmbH
Aluminium can	14 mL; Obtained from Presspart GmbH

### 10.3.5 API - XRPD AND DSC DIAGRAMS

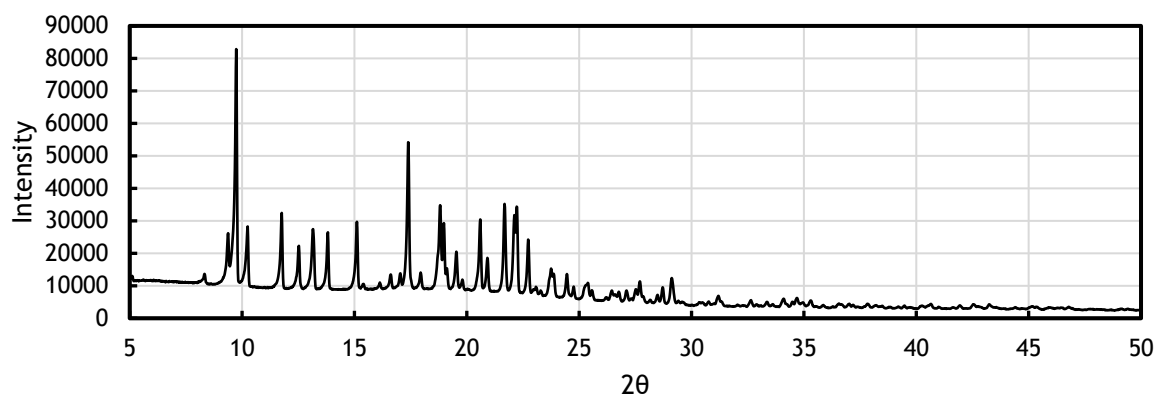


Figure 10–1: X-Ray diffractogram of the CBD extract

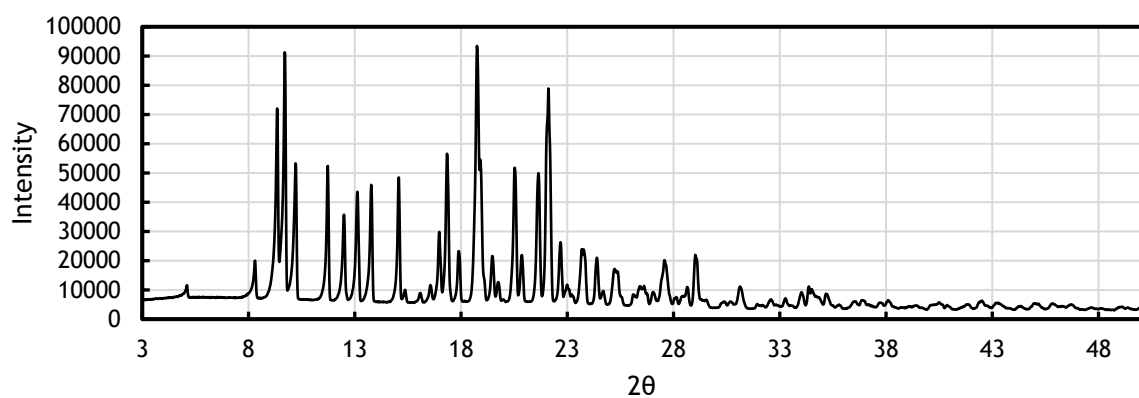


Figure 10–2: X-Ray diffractogram of pure CBD

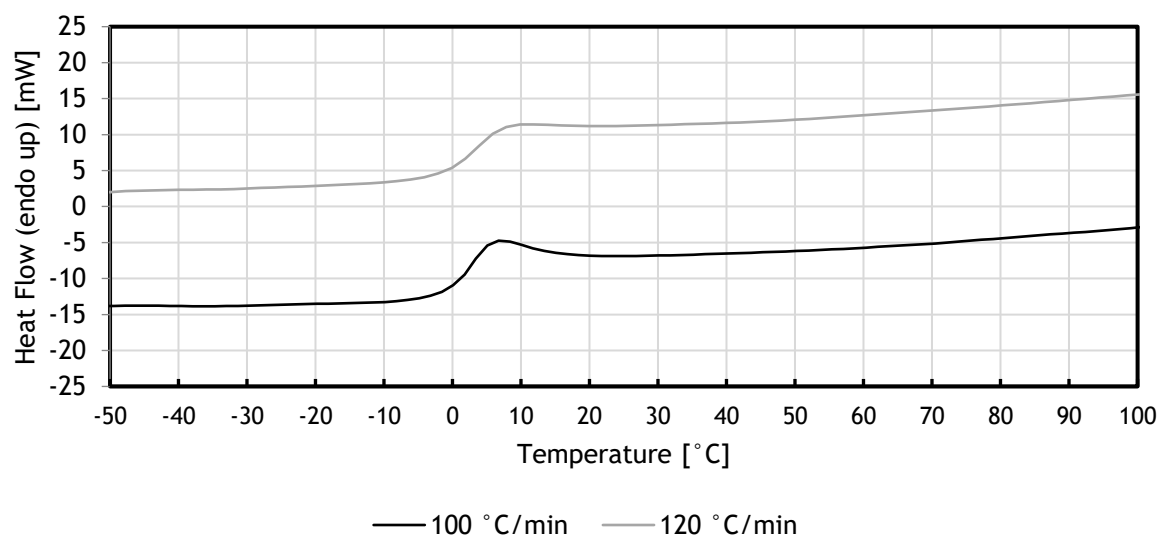


Figure 10–3: HyperDSC® diagram of pure CBD, heating rates 100 °C/min & 120 °C/min; 120 °C/min shifted by +20 MW

## 10.4 HPLC METHODS

### 10.4.1 ISOCRATIC METHOD

Calibration:	0.1 – 100 (250) µg/mL
Column:	RP-18, LichroCART 125-4 with Lichrospher 100 CN (5 µm) precolumn
Detector:	Diode Array Detector (DAD), detection wavelength 220 nm
Flowrate:	1.0 mL/min
Injection volume:	20 µl
Mobile phase:	Acetonitrile:Aq. bidest. (85:15)
Oven temperature:	55 °C
Retention time:	~2.5 min (CBD)
Runtime:	6 minutes
Solvent:	Acetonitrile:Aq. bidest. (85:15)

### 10.4.2 GRADIENT METHOD

Table 10—1: HPLC Gradient, solvent percentage at time, aq. bidest. (A) and acetonitrile (B)

<i>Time [min]</i>	<i>A [%]</i>	<i>B [%]</i>
<i>0</i>	80	20
<i>2</i>	20	80
<i>12</i>	10	80
<i>13</i>	80	20
<i>14</i>	80	20

Calibration:	0.1 – 100 (250) µg/mL
Column:	RP-18, LichroCART 125-4 with Lichrospher 100 CN (5 µm) precolumn
Detector:	Diode Array Detector (DAD), detection wavelength 220 nm
Flowrate:	1.0 mL/min
Injection volume:	20 µl
Mobile phase:	Gradient (Table 10—1), aq. bidest. & acetonitrile
Oven temperature:	30 °C
Retention time:	~6.1 min (CBD)
Runtime:	14 minutes
Solvent (regular):	Acetonitrile:Aq. bidest. (85:15)
Solvent DUSA/NGI:	MeOH:Aq. bidest. (75:25)

## 10.5 EXPERIMENTAL DESIGNS

Table 10–2: Runs for the DoE of the aerodynamic characterisation of the pMDIs

<i>Run Name</i>	<i>Theor. Dose [mg]</i>	<i>Orifice [mm]</i>
<i>N1</i>	0.61	0.22
<i>N2</i>	0.61	0.3
<i>N3</i>	0.61	0.42
<i>N4</i>	1.83	0.22
<i>N5</i>	1.83	0.3
<i>N6</i>	1.83	0.42
<i>N7</i>	3.05	0.22
<i>N8</i>	3.05	0.3
<i>N9</i>	3.05	0.42
<i>N10</i>	0.61	0.22
<i>N11</i>	0.61	0.3
<i>N12</i>	0.61	0.42
<i>N13</i>	1.83	0.22
<i>N14</i>	1.83	0.3
<i>N15</i>	1.83	0.42
<i>N16</i>	3.05	0.22
<i>N17</i>	3.05	0.3
<i>N18</i>	3.05	0.42
<i>N19</i>	0.61	0.22
<i>N20</i>	0.61	0.3
<i>N21</i>	0.61	0.42
<i>N22</i>	1.83	0.22
<i>N23</i>	1.83	0.3
<i>N24</i>	1.83	0.42
<i>N25</i>	3.05	0.22
<i>N26</i>	3.05	0.3
<i>N27</i>	3.05	0.42

## Experimental Designs

Table 10—3: Runs for the HPMC DoE

<i>Run Name</i>	<i>Inlet Temperature [°C]</i>	<i>Solid Content [%]</i>	<i>Aspirator Rate [%]</i>
<i>N1</i>	100	1	50
<i>N2</i>	110	1	50
<i>N3</i>	120	1	50
<i>N4</i>	100	2	50
<i>N5</i>	110	2	50
<i>N6</i>	120	2	50
<i>N7</i>	100	5	50
<i>N8</i>	110	5	50
<i>N9</i>	120	5	50
<i>N10</i>	100	1	75
<i>N11</i>	110	1	75
<i>N12</i>	120	1	75
<i>N13</i>	100	2	75
<i>N14</i>	110	2	75
<i>N15</i>	120	2	75
<i>N16</i>	100	5	75
<i>N17</i>	110	5	75
<i>N18</i>	120	5	75
<i>N19</i>	100	1	100
<i>N20</i>	110	1	100
<i>N21</i>	120	1	100
<i>N22</i>	100	2	100
<i>N23</i>	110	2	100
<i>N24</i>	120	2	100
<i>N25</i>	100	5	100
<i>N26</i>	110	5	100
<i>N27</i>	120	5	100
<i>N28</i>	110	2	75
<i>N29</i>	110	2	75
<i>N30</i>	110	2	75

Table 10–4: Runs for the HPMC-AS DoE

<i>Run Name</i>	<i>Inlet Temperature [°C]</i>	<i>Solid Content [%]</i>	<i>HPMC-AS Quality</i>
<i>N1</i>	70	1	HPMC-AS LF
<i>N2</i>	70	2	HPMC-AS LF
<i>N3</i>	70	5	HPMC-AS LF
<i>N4</i>	100	1	HPMC-AS LF
<i>N5</i>	100	2	HPMC-AS LF
<i>N6</i>	100	5	HPMC-AS LF
<i>N7</i>	130	1	HPMC-AS LF
<i>N8</i>	130	2	HPMC-AS LF
<i>N9</i>	130	5	HPMC-AS LF
<i>N10</i>	70	1	HPMC-AS MF
<i>N11</i>	70	2	HPMC-AS MF
<i>N12</i>	70	5	HPMC-AS MF
<i>N13</i>	100	1	HPMC-AS MF
<i>N14</i>	100	2	HPMC-AS MF
<i>N15</i>	100	5	HPMC-AS MF
<i>N16</i>	130	1	HPMC-AS MF
<i>N17</i>	130	2	HPMC-AS MF
<i>N18</i>	130	5	HPMC-AS MF
<i>N19</i>	100	2	HPMC-AS LF
<i>N20</i>	100	2	HPMC-AS LF
<i>N21</i>	100	2	HPMC-AS LF

## *10.6 CURRICULUM VITAE*

### Personal Data

Name	Paul Friedrich Tobias Gutowski
Date of birth	January 27th 1992
Place of birth	Wedel (Holstein)
Nationality	German

### Work experience

Since July 2016	pharmacist at Citti Park Apotheke Rüdell, Kiel
Since August 2017	pharmaceutical service provider at Pharmatech GmbH
October 2016 – July 2017	Research assistant, Kiel University Supervision of student training in galenics
October 2016 – July 2017	Freelancer for pharmaceutical services
August 2016 – September 2016	pharmaceutical service provider at Pharmatech GmbH

### University Education

Since August 2016	Doctoral candidate at the department of pharmaceutics and biopharmaceutics, Kiel University, Kiel
July 2016	3rd state examination and licensure as pharmacist
December 2015 – May 2016	Pharmacist in training at Citti Park Apotheke Rüdell, Kiel
June 2015 – November 2015	Pharmacist in training at medac GmbH, Wedel
May 2015	2nd state examination
March 2013	1st state examination
April 2011 – May 2015	Pharmacy studies at Kiel University, Kiel

### Alternative civilian service

July 2010 – December 2010	Krankenhaus Tabea, Hamburg
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### Basic and secondary school education

June 2010	Abitur
2001 – 2010	Johann-Rist-Gymnasium, Wedel
1998 – 2001	Altstadtschule, Wedel



## *11 DECLARATION IN LIEU OF OATH*

Hiermit versichere ich, dass ich die vorliegende Dissertation, abgesehen von der Beratung durch den Betreuer, selbstständig verfasst habe und keine weiteren Quellen und Hilfsmittel als die hier angegebenen verwendet habe. Die Dissertation wurde weder in gleicher noch in ähnlicher Form im Rahmen eines Prüfungsverfahrens vorgelegt. Diese Arbeit ist unter Einhaltung der Regeln der guten wissenschaftlichen Praxis der Deutschen Forschungsgemeinschaft entstanden. Außerdem versichere ich, dass mir noch nie ein akademischer Grad ab-erkannt wurde.



Tobias Gutowski

Kiel, 2021

### *DANKSAGUNG*

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man kann sich seine Familie nicht aussuchen und doch sind es im Normalfall die Menschen, die einem besonders nahe sind. Vielen Dank für die schönen Abende, das Vertrauen, das offene Ohr und das unentwegte Aufbauen, wenn wieder einmal etwas nicht so funktioniert hat, wie es sollte.

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- Anna, danke für deinen Humor und deine witzigen Sprüche bei meinen Besuchen in eurem Büro.
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