Comparative Evaluation of Gelatin and Hydroxypropyl Methylcellulose Capsules for Dry Powder Inhalers: Moisture Uptake, Mechanical Integrity, and Implications for Pulmonary Delivery

PhD thesis

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Budapest 2025

1. Introduction

1.1. Pulmonary drug delivery significance and challenges

1.1.1. Introduction to pulmonary drug delivery

In the history of medical advancement, the evolution of drug delivery systems has been critical in improving therapeutic outcomes. From ancient cures to modern pharmaceutical innovations, this path has expanded across multiple routes of administration. In this complex framework, the pulmonary drug delivery in particular emerges with historical significance (with the first inhalation treatment of Hyoscyamus niger dating to approximately 1554 B.C. in ancient Egypt) and future potential (1–4). The development of pulmonary drug administration opens the door to a world in which medicines that have traditionally been administered orally or intravenously find a new way to be delivered. This intricate process involves the targeted administration of therapeutic agents to the lungs, providing a route for fast absorption and local but also systemic circulation: the pulmonary route, primarily known for its role in respiratory conditions, offers an additional and significant aspect of its potential which extends beyond pulmonary diseases. Indeed, over the past decades, inhalation therapy underwent substantial advancements leading to its widely adopted approach to manage respiratory conditions such as asthma and Chronic Obstructive Pulmonary Disease (COPD). The prevalence of modern inhalation devices has played an important role in this progression - particularly Dry Powder Inhalers (DPIs), due to their effective drug delivery potential and unique advantages, as well as an enhanced environmental sustainability when compared to metered-dose inhalers (MDIs) (5–8).

1.1.2. Advantages of pulmonary drug delivery

The pulmonary drug delivery provides distinct advantages in the treatment of respiratory disorders thanks to its targeted delivery approach. It guarantees a rapid drug absorption and deposition at the site of action by delivering medicines directly to the lungs, thus reducing systemic exposure and potential side effects while still enhancing

therapeutic results, patient adherence, and overall quality of life (9,10). Moreover, the non-invasive nature of the inhalation therapy also constitutes a distinctive feature which contributes to its patient-friendly appeal and therapeutic efficiency (9–13).

The progress of both pharmaceutical formulations and inhaling devices over time enabled the pulmonary route to be used for a targeted, local, but also, as mentioned previously, systemic drug delivery. Certain drugs can be effectively absorbed into the bloodstream via the lungs, offering an alternative route for systemic therapy in case of cancer, migraine, diabetes, infections, autoimmune diseases, as well as delivering therapeutic agents such as opiates or anesthetics (9,10,14,15). The pulmonary route is therefore also particularly interesting in case of poorly water-soluble components that demonstrate insufficient bioavailability by other delivery routes, such as the oral route (10–12,17). Indeed, the bypassing of the first-pass metabolism in the liver, potentially increasing the bioavailability of drugs, also constitutes a strategic characteristic which enhances therapeutic efficacy. Moreover, in the pulmonary drug delivery, enhancing the safety profile of a medication also involves the reduction of side effects, emerging as a notable advantage which emphasizes the potential to enhance patient compliance and treatment tolerability. This is attributed not only to a targeted delivery to the lungs, thereby minimizing the likelihood of adverse reactions associated with systemic administration but also to the use of a lower concentration of the drug, further contributing to the reduction of potential adverse effects (9,10,12,18). Indeed, due to its targeted delivery directly to the lungs, pulmonary administration requires a smaller drug dose compared to conventional methods like oral administration, as it reaches the specific site of action more efficiently (10,17,18). Furthermore, the rapid and efficient drug absorption in the lungs is prominently due to their physiological aspects, notably a large absorptive surface area expanding to about 100 m², a high vascularity (5 1/min), a high tissue permeability, and the thin alveolar epithelium, allowing the rapid and efficient absorption of soluble and permeable pharmaceutical compounds (10,12,20,21).

While pulmonary delivery can be achieved through the nasal or oral route, the latter is preferred in terms of drug deposition due to the more favorable physiological structure of the human lungs. Starting from the trachea, the airways branch in a bifurcating manner, diverging into bronchi, respiratory and terminal bronchioles, and finally alveoli. As the

airways progress, their function shifts from conducting air in the larger passages to facilitating gas exchange in the peripheral lung regions. Overall, the intricate structure of the respiratory system, with variations in tube dimensions and a considerable increase in tube numbers, contributes to an extensive internal lung surface area, therefore providing an optimal platform for vital gas exchange.

Nevertheless, the intricate structure of the lungs also poses the primary challenge for pulmonary drug delivery, since it has developed to keep foreign objects from entering the peripheral regions of the lungs. As a result, for inhaled medication delivery to be successful, aerosols must adhere to a rigid set of physical and chemical requirements (12). Other drawbacks also include a typically short duration of activity, resulting from either the rapid elimination of the drug from the lungs or through drug metabolism. Furthermore, while the dose required is minimized, frequent dosing might become a requirement.

1.1.3. Challenges in pulmonary drug delivery

While still quite promising, the pulmonary drug delivery also poses other possible limitations such as the stability of the drug in vivo. Simultaneously, the transport of drugs through the pulmonary route can also become an obstacle, as well as achieving target specificity (17). Additionally, concerns about drug irritation and potential toxicity further underscore the complexity and challenges associated with pulmonary drug delivery. Addressing these limitations is crucial to advance the efficacy and safety of this drug delivery approach.

One of the main challenges faced by the pulmonary drug delivery is the lungs' highly efficient clearing processes designed to prevent undesired environmental particles from infiltrating the respiratory system. Mucociliary clearance is the main mechanism in the upper respiratory tract and involves mucus trapping particles, then cilia moving it out. In addition to the mucociliary clearance mechanism, the deep lung, including the alveoli, features another strong clearance mechanism known as alveolar macrophages. They engulf and digest particles ranging from 1.5 to 3.0 μ m, which became an exploited characteristic while designing inhalable drugs to avoid clearance and enable controlled release within the deep lung. The presence of alveolar macrophages also limits the half-

life of inhalable drugs within the alveoli to a few hours, necessitating increased dosing frequency. Inhaled drugs are also highly susceptible to enzymatic degradation within the lung. Cytochrome P450 (CYP) enzymes, prevalent in the lungs, play a significant role in degrading a wide range of inhaled drugs, impacting their bioavailability. Furthermore, a significant obstacle for inhalation therapeutics is their rapid systemic absorption from the lung. This is primarily attributed to the lung's extensive surface area, excellent epithelial permeability, and abundant blood supply, coupled with the dispersed nature of therapeutic aerosols (9,13,19,21). The cough reflex is also another clearance mechanism worth mentioning: it helps clearing the airways of irritants, mucus, and foreign particles. It is a vital response that helps maintaining protecting the respiratory tract. Although indispensable, these clearance mechanisms can have an unfavorable effect on an inhaled drug's therapeutic effectiveness: in the context of drug delivery, clearance mechanisms can impact the therapeutic effectiveness of inhaled drugs by removing them from the lungs before they can exert their intended effects.

Another significant factor to consider when determining the efficacy of an inhaled dose form is drug deposition in the respiratory system. Airway geometry and humidity play crucial roles in the deposition of particles in the respiratory system. The progressive branching and narrowing of the airways increase the likelihood of particle impaction, particularly for larger particles. Additionally, the high humidity in the lung environment affects the size of aerosol particles. This variability in particle size can significantly impact drug deposition within the lung. As such, understanding these factors is essential for optimizing pulmonary drug delivery, as they present challenges in achieving uniform drug deposition and distribution (19).

Particle size significantly influences drug deposition in the lungs, impacting the effectiveness of inhalation therapy. Upon inhalation, particles deposit through various mechanisms including inertial impaction, sedimentation, and diffusion mainly, but also direct interception, and electrostatic deposition. Larger particles are primarily influenced by inertial impaction and sedimentation, with gravitational sedimentation becoming more prominent in smaller airways.

Particle size is typically described using the mass median aerodynamic diameter (MMAD), which represents the diameter below which 50% of the emitted mass is found.

It is often combined with the geometric standard deviation (GSD) to assess the size distribution. However, while these metrics are commonly used, they do not indicate how much of the dose is converted into an aerosol. The fine particle fraction (FPF) and fine particle dose (FPD) are more informative parameters, which indicate the percentage and mass of the dose, respectively, with an aerodynamic diameter typically below a specified size, often 5 μm or less (12). As seen in Table 1, smaller particles, below 0.5 μm, mainly rely on diffusion for deposition. These particles move through the airways due to Brownian motion, which is the random movement of particles caused by collisions with gas molecules. This movement becomes more pronounced as particle size and flow rate decrease. Similarly to sedimentation, diffusion is influenced by time, and therefore, deposition primarily happens in the peripheral airways. However, due to the brief duration particles spend in the respiratory tract and their random motion, the likelihood of deposition through diffusion is minimal, often resulting in the exhalation of very fine particles instead of deposition (10,12,17).

Table 1. Deposition mechanisms in the lungs according to particle size

Particle Size	Mechanism	Parts of Respiratory Tract
Above 5.0 μm	Inertial impaction	Oropharynx and conducting airways
0.5–5.0 μm	Sedimentation	Bronchi, Bronchioles and Alveoli
0.5–3.0 μm	Sedimentation and Diffusion	Bronem, Bronemores and Thireon
Below 0.5 μm	Diffusion and Brownian motion	Alveolar region

Particles of up to 5 μ m (as well as those in the upper nanometer range) undergo sedimentation, which refers to the gravitational settling of said particles due to their weight. As they travel through the airways, they gradually settle and deposit on the airway walls and surfaces in the bronchioles and alveoli. Additionally, as mentioned, sedimentation is influenced by time: the longer a particle remains in an airway, the greater its likelihood of deposition, which makes this mechanism predominant in the peripheral airways where air velocity is low, similar to the settling velocity, and particles have an

increased residence time. Reduced breathing rates also enhance deposition through sedimentation (10,12).

Particles larger than 5 µm are primarily influenced by impaction due to the high particle inertia in the upper airways where air velocity is high and airflow is turbulent. Due to their substantial mass, the particles struggle to quickly adjust to airflow changes at the airway bifurcations, leading them to collide with the airway walls. The likelihood of impaction grows exponentially with particle size, density, and velocity (10,12,17).

1.2. Technological advances and current state of research, emphasis on nanotechnology

Understanding the importance of particle size and deposition mechanisms provides valuable insights into pulmonary drug delivery, leading to technological advancements that have significantly transformed this field. Nanotechnology, in particular, offers innovative solutions to improve the efficacy and safety of delivering drugs to the lungs. Through the precise engineering of nanoparticles, targeted drug delivery systems can be developed, enhancing the deposition of medications at specific sites within the respiratory system. These advancements not only enhance the targeted delivery of medications but also optimize drug release profiles, improve patient adherence, and minimize potential side effects. Innovative drug formulations, as well as increasingly sophisticated devices to deliver them, are crucial to address the challenges in particle deposition and therapeutic efficacy.

In recent years, the pulmonary drug delivery field has seen significant advancements driven by innovative technologies. For instance, liposomal formulations, generally known to enhance the solubility of poorly soluble drugs and prolong drug release, have been developed to address some of these challenges. Other innovative liposomal formulations for inhalation, such as those containing paclitaxel and doxorubicin, offer a targeted drug delivery to the lungs, minimizing systemic side effects and potentially improving therapeutic outcomes, as demonstrated in clinical trials for lung cancer treatment (30).

While liposomal formulations have shown promise in addressing challenges, recent progress in nanotechnology has further pushed the field of pulmonary drug delivery into innovation. Nanotechnology offers advanced solutions to overcome biological barriers and optimize drug delivery to the lungs. Polymeric nanocarriers, a notable example of nanotechnology in drug delivery, have been engineered to move through mucus layers and bypass bacterial biofilms by using unique properties of nanoparticles, such as their small size and large surface area, thereby enhancing drug penetration and bioavailability within the respiratory system (31).

Solid lipid nanoparticles (SLNs) became a promising, safe nanocarrier option for drugs during the 1990s. They constitute another relevant example since they are nanostructures composed of solid lipid particles stabilized by surfactants in an aqueous environment. These lipid nanoparticles offer several benefits for pulmonary administration, including the potential for deep lung deposition by being integrated into breathable carriers due to their decreased size. Moreover, they facilitate prolonged drug release while exhibiting minimal toxicity (10,32).

Similarly, Nanostructured Lipid Carriers (NLCs) also constitute a great example in nanotechnology. They differ from SLNs in their lipid matrix composition: while SLNs consist of a solid lipid or a blend of solid lipids, NLCs are composed of a blend of solid lipid and oil. This blend creates a less ordered lipid matrix in NLCs, providing more space for active compounds. Consequently, NLCs offer advantages over SLNs such as a higher drug loading capacity and a better stability during storage (10).

In addition to SLNs and NLCs, other nanotechnology-based formulations such as nanocrystals offer a notable solution to address challenges associated with low aqueous solubility, therefore enhancing the bioavailability of numerous drugs. Characterized by their reduced size, nanocrystals present a promising solution to overcome physiological barriers in the lungs and improving drug effectiveness. By enhancing dissolution rates and saturation solubility, nanocrystals have the capacity to extend drug retention in the lungs and enhance therapeutic outcomes (33,34).

Overall, inhalable nano-formulations show significant potential in the pulmonary drug delivery, offering advantages such as enhanced solubility, increased bioavailability, and reduced toxicity in contrast to conventional formulations. However, despite the advancement of fundamental research, the lack of translational research poses challenges for industrialization. While only a limited number of nano-formulations have obtained market approval, ongoing advancements in nanotechnology and inhalation devices

present prospects for future developments. Nevertheless, nanotechnology remains a promising promise in the field of pulmonary drug delivery due to its potential to address challenges, offering innovative solutions for enhanced therapeutic outcomes and improved patient care.

The parallel progress between nanotechnology and inhalation devices helped achieving a new level of precision in drug delivery. As nanotechnology continues to transform drug formulation, inhalers are adapting to incorporate these innovations. This enables the delivery of more personalized treatments, ensuring optimized dosing accuracy and targeted drug release, therefore enhancing patient adherence. The evolution of inhalation devices, driven by the increasing prevalence of chronic respiratory conditions has revolutionized pulmonary drug administration. Inhalation therapy is a standard for maintenance treatment due to its efficacy and reduced systemic side effects. However, patients' adherence to treatment remains a significant issue. To address this challenge, precision and control mechanisms have been integrated into inhalation devices to enable accurate dosing, enhance patient compliance, and overcome the limitations of traditional delivery methods. The integration of smart technologies into inhalation systems further improves drug delivery methods. Inhalers equipped with sensors and feedback mechanisms provide real-time data on patients' usage patterns, therefore offering valuable insights to healthcare professionals. Digital platforms also play a crucial role in patient education and engagement, providing tools like medication diaries and mobile apps to improve doctor-patient collaboration and ultimately, the outcome of respiratory diseases (73,74).

1.3. Capsule materials and their influence on DPI performance

1.3.1. Gelatin: properties and performance as a DPI capsule material

Gelatin is a naturally derived polymer obtained through the partial hydrolysis of collagen, primarily sourced from bovine or porcine connective tissue. Under standard, ambient conditions (15-25 °C, 30-65% RH), gelatin maintains a stable moisture content that keeps the capsules flexible and strong. However, their relatively high equilibrium water content makes them sensitive to environmental humidity fluctuations. If stored or

used in very low-humidity (< 30% RH) environments, gelatin tends to lose moisture, leading to rigidity and brittleness. Inversely, exposure to high humidity can cause gelatin capsules to absorb excess water and become overly soft or sticky (5,77,78). Furthermore, moisture migration from gelatin shells to hygroscopic drug formulations can result in physical changes in the powder, including aggregation or reduced dispersibility (77,79).

In the pharmaceutical industry, gelatin is widely used for hard capsule production due to its film-forming capabilities, biocompatibility, and long-established safety profile. It exhibits favorable mechanical properties such as tensile strength, elasticity, and flexibility, which make it suitable for encapsulating both solid and semi-solid formulations (78,80). The physicochemical properties of gelatin capsules are influenced by factors such as molecular weight distribution (81), the degree of cross-linking (82), and residual moisture content (83).

In the context of DPIs, gelatin capsules have traditionally been used since 1971, due to their availability and compatibility with a wide range of powder formulations (84,85). However, as mentioned, their moisture sensitivity remains a significant limitation. Other concerns include the animal origin of gelatin, which may raise issues related to religious acceptability, risk of transmissible spongiform encephalopathies (TSE), and batch-to-batch variability in physicochemical characteristics (86–88). These factors have motivated the search for alternative capsule materials such as hydroxypropyl methylcellulose (HPMC), which offer enhanced stability and broader acceptability profiles (84,89,90).

1.3.2. HPMC: properties and performance as a DPI capsule material

HPMC, also known as hypromellose, is a semi-synthetic polymer derived from plant cellulose and has emerged as a valuable alternative to gelatin in capsule manufacturing. Chemically, HPMC consists of a cellulose backbone substituted with hydroxypropyl and methyl ether groups, which impart unique physicochemical properties including high chemical stability, low hygroscopicity, and mechanical robustness (91,92). Unlike gelatin, HPMC is cellulose-based rather than protein-based, and it contains significantly less water under equilibrium conditions, typically around 4-6% when stored at 20-25 °C and 30-65% RH (5,77,93). It also does not require plasticizers to remain flexible (94).

Furthermore, HPMC's biocompatibility, non-toxic profile, and absence of animal-derived components have contributed to its adoption in pharmaceutical capsule production, especially where regulatory or ethical considerations limit gelatin use.

In terms of drug compatibility, the chemical inertness of HPMC makes it especially suitable for moisture-sensitive or electrostatically charged formulations. Because of their low water content, HPMC capsules are less likely to transfer moisture to hygroscopic drugs, preserving powder stability during storage (95).

While they are generally more costly than gelatin capsules, HPMC shells are now widely used in modern DPI products, especially where stability and reproducibility are critical. However, they can be more difficult to handle during manufacturing, sometimes requiring equipment adjustments and resulting in higher rejection rates compared to gelatin capsules (89).

In summary, HPMC capsules offer a compelling alternative to gelatin by combining robust mechanical performance with enhanced chemical stability and lower moisture content. Their plant-based origin and regulatory advantages address key ethical and environmental concerns, making them particularly well-suited for modern DPI formulations requiring consistent stability. Despite certain manufacturing challenges, the growing adoption of HPMC reflects a shift towards more versatile and patient-friendly capsule technologies in pulmonary drug delivery.

1.3.3. The role of capsule materials in DPI performance

DPIs enable the direct deposition of medication into the lungs, promoting rapid absorption while limiting systemic exposure: factors that collectively improve therapeutic efficacy, patient compliance, and quality of life (2,21). The increasing preference for DPIs is largely attributed to their propellant-free formulations, which take into consideration environmental concerns related to traditional inhalers and offer enhanced chemical stability over liquid alternatives (4,5,7,96). DPIs are also considered to be user-friendly, requiring minimal coordination during use, which makes them accessible across diverse patient groups, including pediatric and geriatric patients. Their breath-actuated mechanism improves both usability and effectiveness by eliminating the need for auxiliary devices such as spacers (5,97).

Regardless of these benefits, the performance of DPIs remains dependent on several variables, including the physicochemical properties of the formulation (e.g. moisture sensitivity), the inhaler design, and the patient's inhalation technique (97,98). Moreover, patient behavior-related factors can also significantly influence DPIs' efficacy (99). Improper storage can compromise capsule integrity and reduce therapeutic efficacy. Notably, exposure of the capsules to ambient air outside their sealed packaging (e.g. storage of the capsules in pill boxes) has been shown to reduce the FPD by approximately 18% within just 24 hours (100). Additionally, when capsules were subjected to accelerated humidity conditions (40 °C, 75% relative humidity (RH)), a reduction of nearly 50% in FPD was observed for some DPIs (101,102).

In capsule-based DPIs, drug powders are typically filled into hard capsules, which are then punctured in the inhaler device to release the formulation upon inhalation. The reliability and efficiency of DPIs are intrinsically linked to the characteristics of their capsule shells, which interact directly with the powder formulation and serve both to protect it and facilitate its delivery (29). Capsule performance is affected by parameters including material composition, moisture content, and lubrication. Although hard gelatin capsules have been widely used for decades, their sensitivity to humidity remains a major drawback, often weakening shell strength and limiting compatible fill materials. Low moisture can cause gelatin capsules to become brittle, which increases the danger of inhalation. To improve stability and aerosolization, modified capsules with plasticizers and HPMC capsules have been developed. HPMC capsules are of plant origin, have a lower moisture content (4.5–6.5%) than gelatin (13–16%), and do not become brittle. They are less susceptible to moisture-related issues, exhibit more stability, and perform better in puncturing tests. Ultimately, the selection between gelatin and HPMC capsules must be tailored to the specific formulation used.

2. Objectives

The performance of DPIs is critically influenced by the properties and stability of their capsule shells, particularly under elevated humidity conditions that may occur during storage/patient handling. While gelatin and HPMC capsules are both widely used in DPIs, comparative studies investigating their behavior under controlled humidity exposure remain limited, especially with respect to their microstructural and mechanical responses.

The primary aim of this thesis is to advance the understanding of how gelatin and HPMC inhalation capsules respond to high-humidity environments by investigating both their macroscopic and molecular-level changes. This study systematically compares the moisture uptake, mechanical integrity, and structural behavior of gelatin and HPMC capsules following exposure to controlled humidity conditions (25 °C, 75% RH), which simulate possible realistic storage/handling scenarios. The overall goal is to identify material-dependent changes that may affect DPI performance and the protection of moisture-sensitive drug formulations.

To achieve this, the specific objectives were:

- To compare the moisture uptake dynamics of gelatin and HPMC capsules, both quantitatively and qualitatively, in order to understand their respective moisture barrier properties.
- To evaluate how humidity exposure affects capsule mechanical performance, including changes in hardness, deformability, and resistance to puncture - factors critical for DPI device functionality.
- To assess surface property alterations, particularly changes in wettability, which may influence drug adhesion or dispersion during inhalation.
- To explore microstructural changes at the molecular scale using positron annihilation lifetime spectroscopy (PALS), to understand how structural changes at the molecular level influence the physical performance of the capsules.
- To determine how capsule composition (gelatin vs. HPMC) influences performance and protection of moisture-sensitive active pharmaceutical ingredients (APIs) in DPIs.

3. Methods

3.1. Materials and methodology

3.1.1. Preliminary evaluation of commercial DPI capsules

A preliminary evaluation was conducted on commercial DPI capsules from Spiriva® (gelatin, Boehringer Ingelheim) and Braltus® (HPMC, Teva) to assess moisture uptake effects on capsule integrity. Capsules were analyzed immediately after unpacking (day 0) and after seven days stored either at room temperature (20–25 °C) in a pill dispenser or under accelerated conditions (40 °C, 75% RH) in a climate chamber (Memmert Constant climate chamber HPP110ECO, Büchenbach, Germany).

3.1.2. Gelatin and HPMC capsules comparative study

This study used size 0 empty hard capsules of gelatin (Capsugel® Coni-Snap®) and HPMC (Capsugel® Vcaps® Plus) from Lonza (Basel, Switzerland). Capsules were exposed to 25 °C and 75% RH in a climate chamber for 30 min, 1 h, 2 h, 4 h, 8 h, and 24 h. Unexposed capsules stored dry in original packaging served as controls. Both capsule types were analyzed at each time point.

3.2. Experimental methods

3.2.1. Preliminary evaluation of commercial DPI capsules3.2.1.1. Qualitative evaluation of capsule and powder appearance

Visual stability of commercial DPI capsules under different storage conditions was assessed using high-resolution digital microscopy (Keyence VHX-970F, Osaka, Japan). Entire capsules were imaged, focusing on powder morphology and any physical changes in the capsule shell.

3.2.1.2. Scanning Electron Microscopy (SEM)

SEM was used to examine the surface morphology and structural changes of Braltus® capsule powder after 0, 3, and 7 days of high humidity exposure. Powder

samples were collected from capsules, mounted on adhesive tape, and gold-coated using an Emitech K550X Sputter Coater (Quorum Technologies Ltd., Ashford, UK) for 2 min for conductivity and image resolution. SEM imaging (FEI Inspect S50, 20 kV, 21–22 mm working distance) was performed at $300\times$ to $4000\times$ magnification with morphological accuracy within \pm 2%. to assess shape and aggregation changes under moisture stress.

3.2.1.3. Capsule mass variation

The mass of individual capsules from Spiriva® and Braltus® was measured to assess environmental effects on capsule integrity. Ten capsules per product were weighed at day 0, then stored for 7 days either at room temperature in a pill dispenser or under accelerated conditions in a climate chamber. Capsules were reweighed using a high-precision balance (Kern ABJ-NM/ABS-N, Kern & Sohn GmbH, Balingen, Germany) to evaluate mass changes and moisture uptake.

3.2.1.4. Karl Fischer moisture analysis

Water content of the dry powders in Spiriva® (gelatin) and Braltus® (HPMC) capsules was measured by Karl Fischer titration. Three capsules per type were analyzed at day 0, after 7 days at room temperature, and after 7 days under accelerated conditions. Powder was collected by opening the capsule shells, and water content changes were expressed as a percentage of the original sample weight.

3.2.2. Gelatin and HPMC capsules study methods

3.2.2.1. Moisture uptake

Moisture uptake was quantified by weighing 20 individual capsules before and immediately after controlled humidity exposure. Capsules were weighed promptly upon removal from packaging and climate chamber to minimize ambient moisture effects. The mass difference was used to calculate moisture uptake percentage. All measurements were done using a high-precision balance.

3.2.2.2. Visual observations

Moisture absorption by gelatin and HPMC capsules was qualitatively monitored using cobalt(II) chloride silica gel beads (1–4 mm, Kieselgel brown/blue, Merck KGaA, Darmstadt, Germany) that change color from blue (dry) to brown (moist). Beads were oven-dried (AccuDry, ARTEK Systems Corporation, Bothell, WA, USA) at 120 °C for 2 hours before use to ensure dryness. Two reference samples were prepared: oven-dried (dry) and water-soaked (fully saturated). Capsules were filled with dried beads, stored under test conditions, then opened and imaged with a digital microscope to document color changes indicating moisture uptake.

3.2.2.3. Hardness

Capsule hardness was measured using a tablet hardness tester (8M, Dr. Schleuniger® Pharmatron, Solothurn, Switzerland) adapted for size 0 hard capsules. Capsules were placed horizontally between flat metal plates with domes aligned to ensure even force distribution. Increasing pressure was applied until deformation occurred, recording the maximum force in Newtons. Five capsules per type were tested at each storage time point.

3.2.2.4. Moisture analysis

Moisture content of gelatin and HPMC capsules was measured using a moisture analyzer (SCALTEC SMO 01, Scaltec Instruments GmbH, Göttingen, Germany). Capsules were flattened using a tablet hardness tester to ensure even heat distribution and consistent moisture release. Five flattened capsules were analyzed simultaneously at 105 °C. Measurements were taken after humidity exposure, with three replicates per time point, each using five capsules. Moisture content was expressed as a percentage of total sample weight based on weight loss during heating.

3.2.2.5. Wettability

Capsule surface wettability was assessed via contact angle measurement using the sessile drop method. A 100 µL water droplet was deposited onto each capsule surface with a syringe equipped with a fixed needle (Pressure-Lok® Series C-160, Precision Sampling, Baton Rouge, LA, USA, and the contact angle was measured using a digital microscope at 90°. Measurements were performed on five gelatin and five HPMC capsules under dry and 24-hour humid storage conditions.

3.2.2.6. Mechanical analysis

The mechanical behavior of gelatin and HPMC capsules after humidity exposure was assessed using a TexturePro Texture Analyzer (Brookfield CT3-4500, AMETEK Brookfield, Middleborough, MA, USA) with a 4.5 kg load cell. Three tests (horizontal deformation, vertical deformation, and puncture) were performed on five capsules per type and time point. Key parameters included: probe type (12.7 mm diameter TA5 cylinder or 1.0 mm diameter TA9 needle for puncture); probe length (35 mm); test speed (1.00 mm/s for deformations, 0.50 mm/s for puncture), and target distance (4.0 mm for horizontal deformation, 3.0 mm for the vertical one, and 6.0 mm for the puncture). Stiffness (force curve slope) and area under the curve (AUC) were calculated to quantify mechanical strength and flexibility. Capsules were precisely aligned using a custom 3D-printed support using an Original Prusa SL1S Speed 3D printer (Prusa Research a.s., Prague, Czech Republic) to minimize orientation variability during testing.

3.2.2.7. Positron Annihilation Lifetime Spectroscopy (PALS)

PALS was used to assess molecular-level structural changes in gelatin and HPMC capsules due to moisture uptake. Ortho-positronium (o-Ps) lifetime and intensity, along with mean positron lifetime, were measured as indicators of free volume changes. A carrier-free 22 NaCl positron source encapsulated between thin Kapton foils (2 mg/cm²) and possessing an activity of $\sim 5 \times 10^5$ Bq was used. Measurements were performed using a fast–fast coincidence system with two BaF2 detectors, Philips XP2020Q photomultipliers tubes (Koninklijke Philips N.V., Eindhoven, The Netherlands), and

ORTEC electronics, with a time resolution of ~230 ps. Multiple capsules were analyzed per time point to ensure reproducibility.

4. Conclusions

The research presented in this thesis aimed to evaluate and compare the impact of moisture exposure on gelatin and HPMC capsules under conditions relevant to DPI applications. The study focused on understanding how capsule composition influences moisture uptake, structural integrity, and physicochemical behavior, with the goal of comparing and identifying which capsule type is more suitable to protect moisture-sensitive dry powder formulations in high-humidity environments.

To achieve this, gelatin and HPMC capsules were exposed to controlled conditions of 25 °C and 75% RH over multiple time points (30 minutes to 24 hours), with dry capsules serving as reference controls. A comprehensive set of analytical techniques (including gravimetric measurements, silica bead color change, mechanical deformation tests, puncture analysis, surface wettability assessment, and PALS) was used to characterize the capsules' responses to moisture exposure from both macroscopic and molecular perspectives.

Mass gain and water content analyses demonstrated that although HPMC capsules absorbed moisture more rapidly in the early stages of exposure, gelatin capsules ultimately reached higher equilibrium water content, indicating greater hygroscopicity. These findings were visually supported by the progressive color change of cobalt(II) chloride-based silica beads, which occurred more rapidly in gelatin capsules, reflecting their faster internal humidity increase.

Mechanical testing, including hardness measurements and texture analysis (horizontal and vertical deformation, and puncture force), demonstrated that gelatin capsules softened substantially with humidity exposure, with a significant decline in puncture force occurring within the first hour, indicating a rapid loss of structural rigidity. HPMC capsules, while also affected by moisture, retained puncture resistance better over time but exhibited earlier losses in radial stiffness, as observed in horizontal deformation tests.

Surface wettability analysis revealed a statistically significant increase in the contact angle of gelatin capsules after 24 hours of exposure, indicating reduced hydrophilicity. In contrast, HPMC capsules showed only a minor, non-significant increase in contact angle, maintaining more stable surface properties, which are critical for effective powder dispersion.

These findings were corroborated by PALS, which investigated the supramolecular-level behavior of the two materials. HPMC capsules consistently exhibited larger free-volume voids compared to gelatin, which helps explain their faster initial water sorption and suggests greater molecular mobility under humid conditions. Gelatin capsules showed a more monotonic increase in free volume with exposure, indicative of progressive microstructural disruption.

Taken together, the findings demonstrate a fundamental difference in how gelatin and HPMC capsules respond to moisture exposure. Gelatin capsules exhibit faster and more extensive softening, greater structural changes, and a notable decline in mechanical performance. In contrast, HPMC capsules exhibited better retention of physical and mechanical properties under the same conditions.

Importantly, this study highlights that capsule performance is context-dependent. Gelatin capsules may offer advantages in short-term or tightly controlled environments due to their higher lateral stiffness. However, under prolonged humid or tropical storage conditions, HPMC capsules demonstrate superior stability in terms of moisture uptake, mechanical strength, and surface properties, factors that are critical for maintaining dose uniformity and device compatibility in DPI products.

In conclusion, HPMC capsules offer advantages over gelatin capsules in terms of moisture resistance, mechanical stability, and overall consistency under high-humidity storage conditions. These properties make HPMC capsules more suitable for DPI applications, particularly in climates or settings where exposure to humidity is a concern. Future research should further investigate long-term storage behavior, capsule-drug interactions, and performance during inhaler use. Overall, this work contributes valuable data to support an informed selection of capsule materials for moisture-sensitive pulmonary drug delivery systems.

5. Bibliography of the candidate's publications

5.1. Related to the thesis

Magramane S, Kállai-Szabó N, Farkas D, Süvegh K, Zelkó R, Antal I. Comparative Evaluation of Gelatin and HPMC Inhalation Capsule Shells Exposed to Simulated Humidity Conditions. Pharmaceutics. 2025 Jul 03;17(7):877.

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5.2. Other Publications

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