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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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List of Abbreviations

ACI – Andersen Cascade Impactor

AUC – Area Under the Curve

API – Active Pharmaceutical Ingredient

COPD – Chronic Obstructive Pulmonary Disease

DPI – Dry Powder Inhaler

FDA – Food and Drug Administration

FPD – Fine Particle Dose

FPF – Fine Particle Fraction

GSD – Geometric Standard Deviation

HPMC – Hydroxypropyl Methylcellulose

LF-MRI – Low-Field Magnetic Resonance Imaging

LOD – Loss On Drying

MDI – Metered-Dose Inhaler

MMAD – Mass Median Aerodynamic Diameter

NGI – Next Generation Impactor

NLCs – Nanostructured Lipid Carriers

o-Ps – ortho-positronium

PALS – Positron Annihilation Lifetime Spectroscopy

RH – Relative Humidity

RT – Room Temperature

SD – Standard Deviation

SEM – Scanning Electron Microscopy

SLNs – Solid lipid nanoparticles

Tg – glass transition temperature

TSE – Transmissible Spongiform Encephalopathies

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). Other therapeutic agents such as proteins and peptides (insulin for instance) in particular demonstrate varying degrees of systemic bioavailability when administered via inhalation, which is significant since they cannot be delivered systemically without invasive methods, and are susceptible to be broken down by metabolic enzymes (e.g. pepsin) when administered gastrointestinally (11,12,14–16). 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. Delivering the compounds directly avoids initial liver metabolism, allowing a significant portion of the compound to reach the intended organ, all while maintaining controlled systemic exposure (9,10,17). As it circulates throughout the bloodstream, a drug can create systemic toxicity: a harmful undesired effect. In the context of pulmonary drug delivery, a reduced systemic toxicity means that a smaller portion of the drug enters the systemic circulation, leading to fewer harmful effects on organs and tissues outside the respiratory system. Therefore, similarly to bypassing first pass metabolism, the pulmonary delivery allows for the avoidance of systemic toxicity as well, which constitutes a notable benefit (9,10,13). 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 (such as gastrointestinal issues: gut irritability, undesired

metabolites, food-related effects (17)) 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). For instance, the drug content in a single 4 mg tablet of salbutamol is equivalent to 40 measured inhalations, meaning that a minimal fraction of the oral dose is needed to achieve the same desired therapeutic effect (17,19). This is possible because of the targeted nature of the drug delivery, which allows for a rapid onset of action (10,12,17–19). For instance, in the treatment of breakthrough pain in cancer patients with fentanyl, a high level of satisfaction was reported, with an average time to pain relief of 12 minutes (15). 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 m^2, a high vascularity (5 l/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. The respiratory tract comprises two functional parts: the conducting airways and the respiratory zone. The simplest and most frequently used lung model is Weibel's lung model, which divides the lung into 24 parts or generations (G): the trachea (G0), and 23 consequent generations (G1 to G23), as shown on Figure 1 (10,22,23).

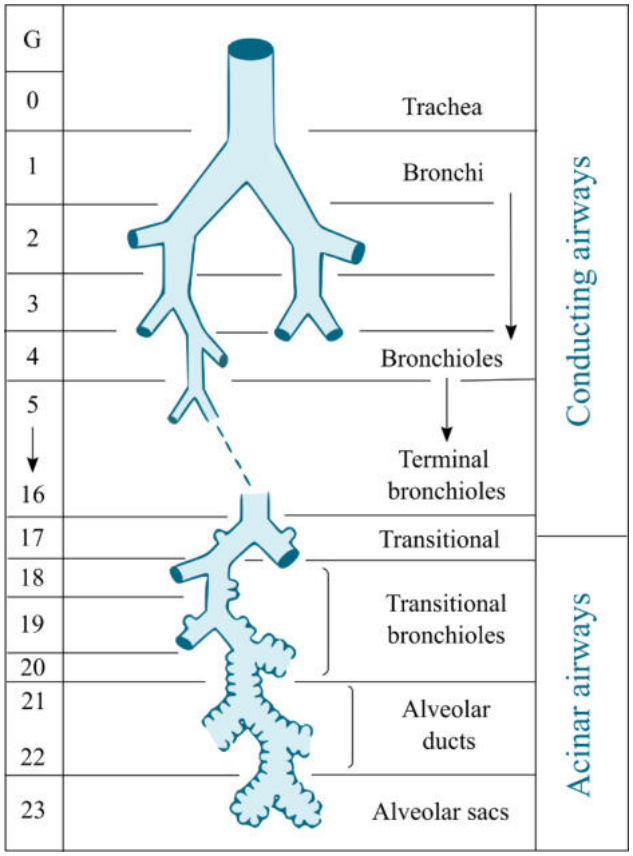

Figure 1. Weibel's lung model (based on (22,24))

From the trachea (G0) to the alveolar ducts (G22), decreasing tube length and cross-sectional tube area can be observed, though accompanied by an increasing number of tubes. The trachea's diameter is approximately 2.5 cm while that of the alveolar ducts is between 0.2 and 0.5 mm. Altogether, Weibel reported that the internal lung surface area is in the order of 130 m^2, with the alveoli (about 300 million in an adult lung) covering a surface of more than 100 m^2 (24). In the conducting zone, the mouth, nasal cavities, as well as the pharynx and larynx efficiently carry the gas to the site of gas exchange while simultaneously filtering, warming, and moistening the incoming air entering the respiratory system. The gas exchange happens over 7 generations, from the bronchioles (G17) to the alveolar sacs (G23) (23). 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 μm, 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 (as seen on Figure 2) 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.

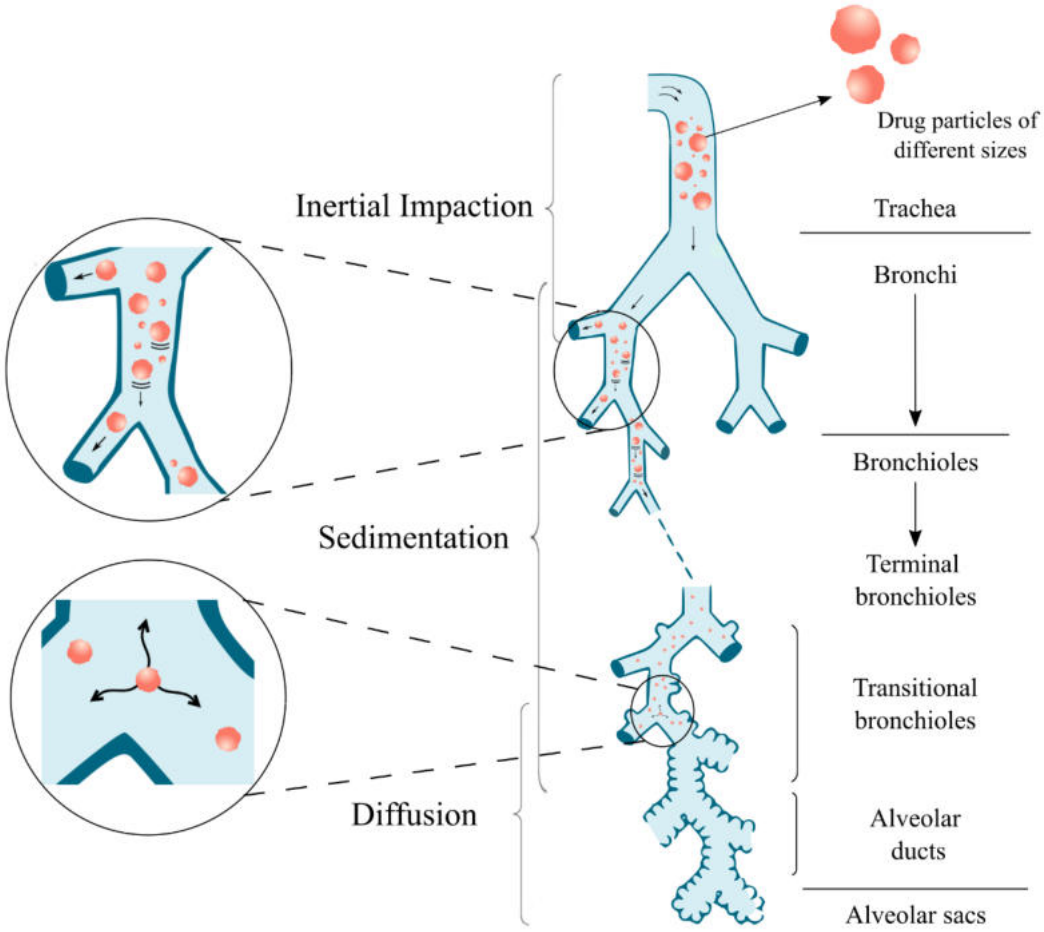

Figure 2. Particle deposition mechanisms according to particle size (25)

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	
Below 0.5 µm	Diffusion and Brownian motion	Alveolar region

Particles of up to 5 µm (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).

Overall, particles with an MMAD between 5 and 10 µm deposit in the oropharyngeal region and large airways, while those between 1 and 5 µm target the small airways and alveoli. Notably, over 50% of 3 µm particles deposit in the alveolar region, whereas

particles smaller than 3 μm have an approximately 80% chance of depositing in the lower airways and a 50–60% chance of reaching the alveoli. Thus, an ideal particle size for effective deposition is around 3 μm (19,23,25,26). Very small particles, less than 1 μm, are equally likely to deposit in the alveoli or be exhaled (27).

In order to accurately assess the deposition of particles in the respiratory system, Cascade Impactors constitute a great method. They are widely used in pharmaceutical studies for measuring particle size distribution of inhaled formulations. These devices, detailed in pharmacopeias, are valued for their ability to directly measure aerodynamic particle sizes and quantify drug mass across various size ranges, minimizing interference from excipients. The Next Generation Impactor (NGI), which has largely replaced the older Andersen Cascade Impactor (ACI), offers advanced particle separation through a series of nozzles and stages, including a micro-orifice collector for capturing very fine particles. This setup allows for precise aerodynamic analysis essential for evaluating inhaler performance (16).

The influence of aerosol particle size on the therapeutic effectiveness was notably shown with the β-agonist salbutamol: dose-response curves revealed that the smaller particle aerosol used (3.3 μm) generated a more significant bronchodilator response across all doses compared to the larger (7.7 μm) particle size aerosol (19). Moreover, another study conducted by Zanen et al. showed that no significant changes in lung function were observed with the placebo or between the bronchodilator responses of salbutamol and ipratropium bromide when the particle sizes were identical. However, significant differences in lung function parameters were found when using different particle sizes (1.5 μm, 2.8 μm, and 5.0 μm). For both ipratropium bromide and salbutamol, an ideal particle size for effective aerosol deposition was around 3 μm. This particle size resulted in the highest bronchodilator response, likely due to the filtering characteristics of the upper airways favoring smaller particles. Lower dosages of these bronchodilators were effective, suggesting that a high percentage of the inhaled dose reached the airways. Thus, the study concludes that a particle size of approximately 3 μm is optimal for bronchodilator aerosols in patients with severe airflow obstruction (28).

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. Formulations designed to address challenges such as mucociliary clearance and variability in lung physiology have been a focus of pulmonary drug delivery. 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. As of 2020, 21 clinical trials were ongoing for liposomal formulations, covering conditions like cystic fibrosis, cancer, and fungal infections. Among these, Arikayce® accounted for the highest number of trials (5 trials) focused on cystic fibrosis, followed by Ambisome® (4 trials) for bronchopulmonary aspergillosis (29). 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). SLNs were developed and optimized as carriers for pulmonary delivery of insulin, resulted in significant reduction of fasting plasma glucose levels in rats, along with uniform distribution in lung alveoli and sustained insulin release, highlighting their promise for systemic protein delivery (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).

Table 2 compiles various nanoparticle types relevant to nanotechnology, including those discussed and additional examples, along with the respective active ingredients used in their formulations.

Table 2. Nanoparticles formulated for dry powder inhalation (based on 25)

Type of nanoparticles	Active ingredient	Ref.
Liposomes	Synergistic Ciprofloxacin and Colistin	(35)
	Ciprofloxacin	(36)
	Isoniazid	(37)
	Insulin	(38)
	Oseltamivir phosphate	(39)
	Curcumin	(40,41)
	Gemcitabine hydrochloride	(42)
	Salbutamol sulfate	(43,44)
	Rifampicin	(45)
	Tacrolimus	(46)
	Dapsone	(47)
Proliposomes	Rifapentine	(48)
SLNs	Insulin	(49)
	Budesonide	(50)
	Alendronate	(51)
	Amikacin	(52)
	Doxorubicin	(53)
	Rifampicin	(54)
NLCs	Montelukast	(55)
	Paclitaxel	(56)
Nanocrystals	Curcumin	(57,58)
	Baicalein	(59)
Polymeric nanoparticles	Sildenafil	(60)
	N-acetylcysteine	(61)
	Heparin	(62)
	Fisetin	(63)
Nanocomposite particles	Curcumin	(64)
	Salvianolic acids	(65)
	Andrographolide	(66)
Porous nanoparticle-aggregate particles	Rifampicin	(67)
	Levofloxacin	(68)
Nanoparticle agglomerates	Nifedipine	(69)
Protein-based nanoparticles	Apigenin	(70)

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. As of now, the Food and Drug Administration (FDA) has only approved one inhaled nano-formulation for marketing: Arikayce® (71). 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.

Moreover, combining pulmonary drug delivery with biotechnology and pharmacogenomics also offers a way to develop innovative drug carriers and targeted delivery systems. An example of that is gene therapy, utilizing nanoparticles (to deliver large genes and plasmids), lipofection (using lipid-based complexes for gene delivery), or electroporation (creating transient pores in cell membranes for gene uptake) (72).

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). Smart inhalers fall into two categories: original integrated and add-on devices. Original integrated devices, such as the Electronic Breezhaler®, come with built-in smart features, while add-on devices can transform traditional inhalers into smart ones. Examples of add-on devices include CareTRx™ and SmartTrack, which have shown to increase medication adherence significantly (by 180% for Smart Track) (75). However, there are several considerations to address before the consideration of a widespread adoption among which (76):

- *Data security and privacy:* ensuring that the patients' data is securely managed and complies with regulations.
- *Acceptance:* gaining the trust of both healthcare providers and patients is a crucial element for the implementation of these technologies.
- *Cost-effectiveness:* evaluating the economic benefits of these devices compared to traditional treatments as this is an important factor.

Overall, recent advancements in pulmonary drug delivery, including innovative formulations and sophisticated delivery devices, address existing challenges and pave the way for more effective and patient-friendly treatment options.

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. Hard gelatin capsule shells are typically formed by dipping stainless-steel pins into a warm gelatin solution (45-55°C, with a set viscosity), then allowing the coated film to dry; the resulting shell contains a large amount of bound water (about 13-16% by weight) which acts as a natural plasticizer and provides structural flexibility. 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 (e.g., in halal or kosher contexts), 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).

In conclusion, while gelatin's established film-forming capabilities, biocompatibility, and strong mechanical properties have secured its place in DPI capsule applications for over five decades, its pronounced sensitivity to moisture and animal-derived origin impose practical and ethical constraints. As a result, ongoing innovation in capsule technology is increasingly favoring synthetic or plant-based polymers (such as HPMC) that deliver comparable performance with greater environmental tolerance and broader patient acceptance.

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. HPMC capsules are produced using either thermal gelation or cold-gelling techniques. Thermal methods involve hot HPMC solutions forming a gel upon cooling, while cold-gelling processes incorporate gelling agents such as carrageenan or gellan gum, often with potassium chloride. The resulting shells are structurally stable, nearly amorphous, and exhibit consistent performance across a wide range of humidity conditions (5).

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. A 2016 study involving 738 participants revealed that nearly two-thirds had not been adequately instructed on proper storage practices (by doctors, nurses, or pharmacists) (99). Improper storage, such as removing capsules from their original blister packaging, 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). A study by Ding et al. demonstrated that the capsule material type has a notable impact on the performance of carrier-free DPI formulations (while only minor differences were observed for carrier-based systems) (103). The study also showed that capsule hardness affects flap detachment during piercing, which in turn influences aerosol performance, highlighting the importance of selecting suitable capsule types for an optimal performance. Buttini et al. similarly emphasized the critical role of capsules in inhalation therapy, identifying essential criteria such as easy puncturing, minimal shell fragmentation, efficient flap opening for powder release, and low powder residual content (5). 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.

In light of these considerations, a deeper understanding of capsule behavior under environmental stress is essential for the development of reliable DPI systems.

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.

Altogether, these objectives aim to inform capsule material selection and handling strategies for DPIs, while contributing to a deeper understanding of capsule performance under humidity stress in pharmaceutical applications.

3. Methods

3.1. Materials and methodology

3.1.1. Preliminary evaluation of commercial DPI capsules

Prior to the main experimental investigation, a preliminary evaluation was conducted on commercial DPI capsules from marketed products: Spiriva® (gelatin-based capsules, API: tiotropium bromide monohydrate, Boehringer Ingelheim), and Braltus® (HPMC-based capsules, API: tiotropium bromide monohydrate, Teva). This preliminary study provided an initial understanding of how moisture uptake impacts capsule integrity in commercially available DPI products, highlighting its practical relevance in real-world storage and handling scenarios.

The capsules were examined immediately after removal from their original packaging (day 0) to characterize their initial condition prior to any environmental exposure. Additional assessments were performed after storage for seven days under two different conditions. One set was stored at room temperature (RT), approximately 20 to 25 °C, inside a pill dispenser to simulate typical patient storage conditions. The other set was stored in a climate chamber (Memmert Constant climate chamber HPP110ECO, Büchenbach, Germany) maintained at 40 °C and 75% RH to simulate accelerated aging and stress conditions.

3.1.2. Gelatin and HPMC capsules comparative study

This study was conducted using size 0 empty hard capsules composed of either gelatin or HPMC. The gelatin capsules (Capsugel® Coni-Snap®) and the HPMC capsules (Capsugel® Vcaps® Plus) were both obtained from Lonza (Basel, Switzerland). To assess the influence of humidity on their properties, the capsules were subjected to controlled environmental conditions: they were placed in a climate chamber (Memmert Constant climate chamber HPP110ECO, Büchenbach, Germany) set to 25 °C and 75% RH.

The capsules were exposed to these conditions for predefined durations: 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, and 24 hours. For each time point, both gelatin and

HPMC capsules were analyzed, and capsules that were not subjected to humidity exposure (dry/0 hours) served as a reference for comparison and were stored in their original packaging, under ambient, dry conditions until use.

3.2. Experimental methods

3.2.1. Preliminary evaluation of commercial DPI capsules

3.2.1.1. Qualitative evaluation of capsule and powder appearance

To qualitatively assess the visual stability of commercially available DPI capsules under varying environmental conditions, high-resolution digital microscopy was used. The capsules were examined under the three different storage conditions. Images were acquired using a digital microscope (Keyence VHX-970F, Keyence Corp., Osaka, Japan). Each capsule was imaged in its entirety, with particular attention paid to the powder morphology and any observable physical changes in the capsule shell.

3.2.1.2. Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) was used to characterize the surface morphology and structural characteristics of the DPI formulation contained in Braltus® capsules and to assess the effects of high humidity exposure after 3 and 7 days. This analysis provided insight into differences in shape, and aggregation tendencies, particularly under moisture stress.

SEM imaging was first performed on the Braltus® capsule powder formulation immediately after removal from the original packaging to assess its initial morphology. Additional SEM analyses were conducted on powders stored inside capsules for 3 and 7 days under accelerated conditions in a climate chamber to evaluate structural changes induced by environmental stress

In each case, the capsule contents were emptied onto a sample holder and fixed using double-sided adhesive tape. The powder samples were gold-coated using an Emitech K550X Sputter Coater (Quorum Technologies Ltd., Ashford, UK) for 2 minutes to ensure adequate conductivity and image resolution. SEM analysis was performed using a scanning electron microscope (FEI Inspect S50) operated at an accelerating voltage of 20.00 kV. The working distance during analysis ranged from 21 to 22 mm. Images were

captured at magnifications ranging from 300× to 4000×, with morphological accuracy within ± 2%.

3.2.1.3. Capsule mass variation

To investigate the influence of environmental exposure on capsule integrity and formulation stability, changes in the mass of individual capsules were measured. Capsules from two marketed DPIs – Spiriva® and Braltus® – were evaluated.

Ten capsules from each product were removed from their original packaging and immediately weighed on day 0 to record their initial mass. Subsequently, the same capsules were stored under two separate conditions: in a pill dispenser at RT for 7 days, and in a controlled climate chamber for 7 days to simulate accelerated conditions. After each storage period, the capsules were reweighed to assess changes in mass. A high-precision analytical balance (Kern ABJ-NM/ABS-N, Kern & Sohn GmbH, Balingen, Germany) was used for all the measurements. The difference in mass over time was used to estimate potential moisture uptake and evaluate the interaction between the capsule shell, its contents, and the surrounding environment.

3.2.1.4. Karl Fischer moisture analysis

The water content of the dry powder formulations contained within Spiriva® (gelatin-based) and Braltus® (HPMC-based) capsules was analyzed using a Karl Fischer titrator (787 KF Titrino, Metrohm AG, Herisau, Switzerland). Three capsules per type were analyzed on day 0 (immediately out of their packaging), after 7 days at RT in a pill dispenser, and after 7 days in a climate chamber. For each condition, the capsule shells were carefully opened and the internal powder was collected for analysis. Changes in water content were expressed as percentages relative to the original sample weight.

3.2.2. Gelatin and HPMC capsules study methods

3.2.2.1. Moisture uptake

To quantify the extent of moisture uptake, the mass of individual capsules was measured before and after exposure to controlled humidity conditions. The capsules were removed from their original packaging and weighed immediately to minimize the influence of ambient humidity. Once the humidity exposure period was completed, the capsules were reweighed as quickly as possible upon removal from the climate chamber to avoid moisture loss during handling.

For each time point (including the unexposed dry reference) a total of 20 capsules were weighed individually. The difference in mass before and after exposure was used to calculate the percentage of moisture uptake for each capsule.

All mass measurements were conducted using a high-precision analytical balance (Kern ABJ-NM/ABS-N, Kern & Sohn GmbH, Balingen, Germany).

3.2.2.2. Visual observations

To monitor the extent of moisture absorption by gelatin and HPMC capsules in a qualitative manner, color-changing silica gel beads (Kieselgel brown/blue, Merck KGaA, Darmstadt, Germany) were used as a visual indicator. These beads, which range in size from approximately 1 to 4 mm in diameter, incorporate cobalt(II) chloride, which undergoes a distinct color transition depending on the moisture content. In their anhydrous (dry) state, the beads appear blue, while upon moisture exposure they turn brown, thereby serving as a reliable visual marker of humidity uptake. Prior to their use in the experiment, the silica beads were dehydrated by placing them in a laboratory oven (AccuDry, ARTEK Systems Corporation, Bothell, WA, USA) at 120 °C for approximately two hours. This step was necessary to ensure that the beads were completely dry and would accurately reflect any subsequent moisture uptake during storage.

Moreover, two types of reference samples were prepared for comparative purposes: one representing the dry condition (using oven-dried beads) and another representing the

fully saturated state, achieved by soaking the beads in water until they reached maximum moisture capacity.

Gelatin and HPMC capsules were filled with the oven-dried silica beads and then subjected to the defined storage conditions. Following the exposure period, the capsules were carefully opened, and the internal silica beads were immediately photographed using a digital microscope (Keyence VHX-970F, Keyence Corp., Osaka, Japan) in order to capture the moisture-induced color change.

3.2.2.3. Hardness

The capsule hardness was measured using a tablet hardness tester (8M, Dr. Schleuniger® Pharmatron, Solothurn, Switzerland), a device widely used in pharmaceutical research and quality control to assess the mechanical strength of solid oral dosage forms, such as tablets and, in adapted setups, capsules. In this study, the apparatus was adapted for use with size 0 hard capsules to evaluate their resistance to compressive force.

Each capsule was positioned horizontally within the device, oriented perpendicularly to the instrument's flat metal compression plates. The capsules were carefully positioned with their domes aligned against the compression plates to ensure an even force distribution. The device then applied increasing pressure until the capsule structure deformed or yielded, allowing the maximum force applied to be recorded.

For each storage time point investigated, five individual capsules of each type were tested. The hardness was defined as the force required to compress/deform the capsule, with results were expressed in Newtons (N).

3.2.2.4. Moisture analysis

The determination of the moisture content within the gelatin and HPMC capsules was carried out using a moisture analyzer (SCALTEC SMO 01, Scaltec Instruments GmbH, Göttingen, Germany).

To improve the accuracy and reproducibility of the moisture content measurements, each capsule was flattened prior to analysis. Flattening the capsules helped ensure even heat distribution and consistent moisture release across the entire shell surface during the

measurement process. This flattening was achieved by placing each capsule individually in a tablet hardness tester, orienting it parallel to the machine's flat metal plates with the capsule length aligned along these surfaces. The device then applied compressive force to the capsule, transforming its original cylindrical shape into a flat, oval form suitable for moisture analysis. Following this, five flattened capsules were simultaneously placed in the moisture analyzer for each measurement.

The moisture analyzer was set to a constant temperature of 105 °C, a widely used setting for moisture determination in pharmaceutical materials where thermal degradation is not a concern. The moisture content was quantified based on the weight loss observed during the heating process, which corresponds to the evaporation of water from the samples.

Measurements were performed after the capsules had been exposed to the humid conditions. For every time point evaluated, three parallel measurements were conducted. Each replicate involved testing five capsules of either gelatin or HPMC simultaneously, and the resulting moisture content was directly recorded as a percentage of the total sample weight.

3.2.2.5. Wettability

The wettability of the capsules' surfaces was assessed by measuring the contact angle using the sessile drop technique, a widely accepted method for evaluating surface hydrophilicity or hydrophobicity. For this procedure, a 100 µL syringe equipped with a fixed needle (Pressure-Lok® Series C-160, Precision Sampling, Baton Rouge, LA, USA) was used to carefully deposit a single droplet of purified water (*aqua purificata*) onto the surface of each capsule. The contact angle was then measured using a digital microscope positioned at a 90° angle relative to the capsule surface. The procedure was applied to both gelatin and HPMC capsules under two conditions: unexposed (dry) capsules and capsules that had been stored for 24 hours in the humid conditions. For each condition and capsule type, measurements were conducted on five individual capsules to ensure reproducibility and to account for sample variability.

3.2.2.6. Mechanical analysis

The mechanical behavior of gelatin and HPMC capsules following exposure to controlled humidity conditions was evaluated using texture analysis. A TexturePro Texture Analyzer (Brookfield CT3-4500, AMETEK Brookfield, Middleborough, MA, USA), equipped with a 4.5 kg load cell, was used to assess the capsules' resistance to external forces. This evaluation aimed to characterize how moisture uptake influenced the capsules' structural integrity, deformation characteristics, and puncture resistance.

Three mechanical tests were carried out: horizontal deformation, vertical deformation, and puncture testing. For every time point and capsule type, five individual capsules were analyzed. The parameters used for each test are detailed in Table 3.

Table 3. Parameters used for the mechanical testing of DPI capsules using a TexturePro texture analyzer

Parameter	**Horizontal deformation**	**Vertical deformation**	**Puncture test**
Capsule orientation	Horizontal	Vertical	Horizontal
Probe type	TA5 cylinder	TA5 cylinder	TA9 needle
Probe diameter (mm)	12.7	12.7	1.0
Probe length (mm)	35	35	35
Test speed (mm/s)	1.00	1.00	0.50
Target distance (mm)	4.0	3.0	6.0
Objective	To measure deformation from side compression; assess lateral structural integrity.	To evaluate capsule's vertical deformation response under downward pressure.	To measure the force required to puncture the capsule.

In addition, two quantitative parameters were also derived from each measurement:

- The stiffness, which describes the slope of the force curve;
- The area under the curve (AUC), which provides an integrated measure of the total mechanical work required during deformation or puncture.

These parameters provided a comparative evaluation of the mechanical strength and flexibility of the capsules across different material types and exposure durations.

To ensure a consistent and precise alignment of the capsules during testing, a custom-made 3D-printed support was used. This support was fabricated using an Original Prusa SL1S Speed 3D printer (Prusa Research a.s., Prague, Czech Republic). It was printed with Prusament Tough Resin in Prusa Orange. As illustrated in Figure 3, the support allowed for accurate horizontal or vertical placement of the capsules during testing, in order to reduce variability related to capsule orientation.

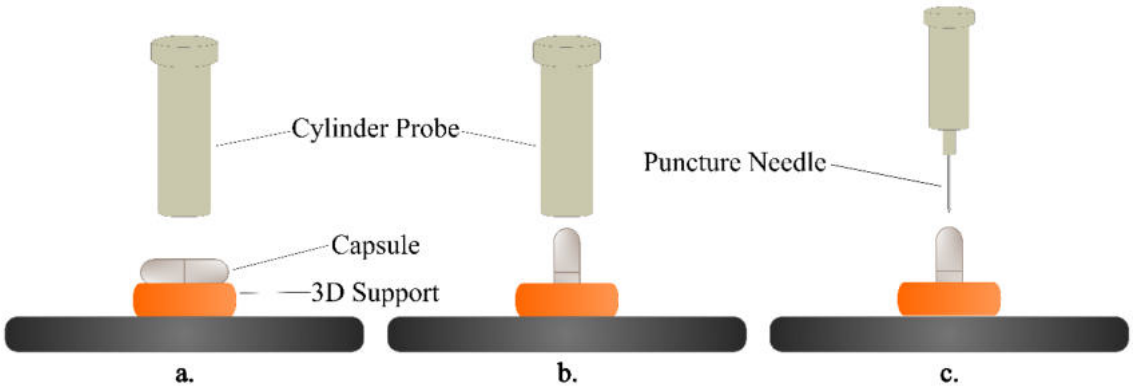

Figure 3. Setup used for the mechanical testing of the capsules via texture analysis: (**a**) horizontal deformation, (**b**) vertical deformation, and (**c**) puncture test

3.2.2.7. Positron Annihilation Lifetime Spectroscopy (PALS)

To investigate changes in the microstructural properties of gelatin and HPMC capsules at the molecular level, PALS analysis was used. This technique provides information on molecular-level structural changes by measuring the lifetime and intensity of ortho-positronium (o-Ps), parameters that are sensitive to the size and distribution of free volume in polymeric materials.

For each sample, the o-Ps lifetime and intensity were recorded to detect changes related to the moisture uptake in the polymers' microstructure. In addition, the mean positron lifetime was evaluated as an overall indicator of changes in the free volume properties across the capsule samples.

The positron source used in these measurements was a carrier-free $^{22}NaCl$ source, encapsulated between thin Kapton foils (2 mg/cm^2) and possessing an activity of approximately 5×10^5 Bq. The measurements were conducted using a fast–fast coincidence system equipped with two BaF_2 crystal detectors and Philips XP2020Q photomultiplier tubes (Koninklijke Philips N.V., Eindhoven, The Netherlands). Standard ORTEC electronics were used for signal processing. The time resolution of the system was approximately 230 ps. To ensure reproducibility, multiple capsules were analyzed at each time point.

4. Results

4.1. Preliminary evaluation of commercial DPI capsules

4.1.1. Qualitative evaluation of capsule and powder appearance

The physical appearance of two commercial DPI products capsules (Spiriva® and Braltus®) was monitored to assess visible changes in capsule and powder characteristics following exposure to different environmental conditions (Figure 4).

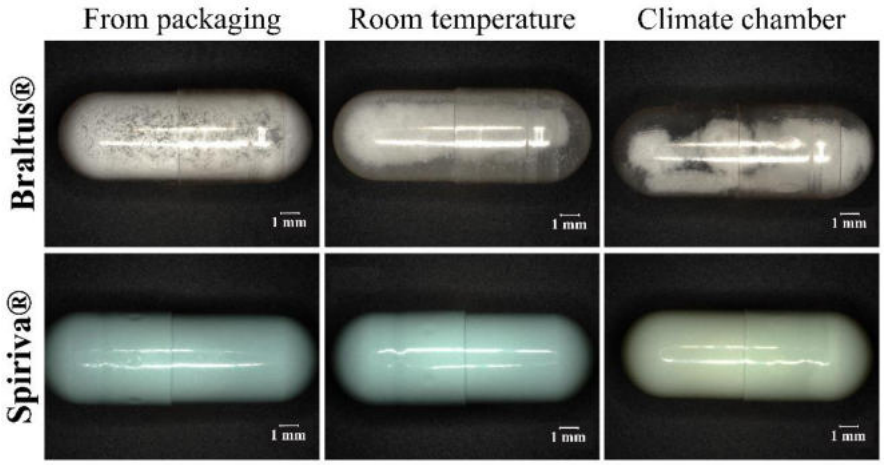

Figure 4. Comparative visual evaluation of commercial DPI capsules (Spiriva® and Braltus®) after removal from original packaging, stored at RT in a pill dispenser for 7 days, and stored in a climate chamber at 40 °C, 75% RH for 7 days

Braltus® DPI capsules, once removed from their packaging and stored at RT for seven days, showed clear signs of powder aggregation, likely due to moisture-induced cohesion. This effect intensified when the capsules were subjected to stress conditions, indicating that the formulation becomes even more prone to moisture-induced clumping under elevated temperature and humidity. In contrast, the opaque Spiriva® capsules did not allow visual assessment of the powder inside; however, a noticeable discoloration of the capsule shell was observed following exposure to 40 °C and 75 % RH. This external change suggests that the capsule material itself may be sensitive to elevated temperature and humidity, serving as an indirect sign of compromised stability.

4.1.2. Scanning Electron Microscopy (SEM)

SEM images of the Braltus® capsule powder formulation are presented in Figure 5.

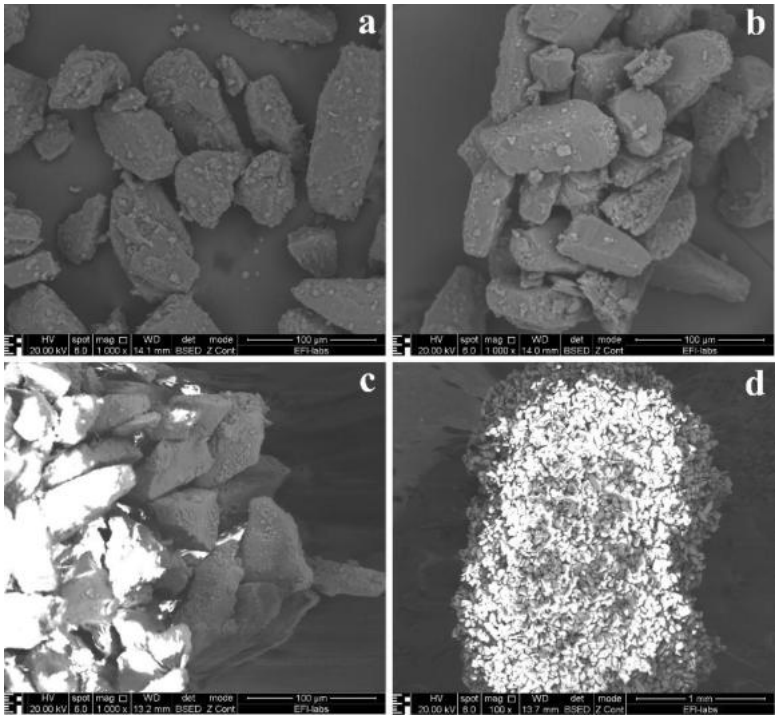

Figure 5. SEM images of Braltus® capsule powder formulation taken directly from packaging (a) and following 3 (b) and 7 (c, d) days of storage in a climate chamber at 40 °C, 75% RH. Magnification levels: (a, b, c) 1000×; (d) 100×

Image (a) shows the powder morphology directly after removal from the original packaging, with distinct and well-dispersed particles. After 3 days in controlled conditions, the powder (image (b)) appears more compacted, with particles showing increased surface contact and initial signs of aggregation. This trend becomes more pronounced after 7 days, as seen in images (c) and (d), where particle clustering and close packing are evident. In particular, image (d), taken at lower magnification, reveals extensive aggregation and the formation of a large cohesive mass, indicating a substantial reduction in particle separation over time.

4.1.3. Capsule mass variation

The mass changes of the capsules were monitored over time to assess their response to different storage conditions. Figure 6 presents the changes in capsule mass observed over a 7-day storage period in a pill dispenser, under standard RT conditions. Both capsule types showed similar results: Spiriva® capsules showed the greatest increase, with mass rising by approximately 5.1%, while Braltus® capsules exhibited an increase of about 5.0%.

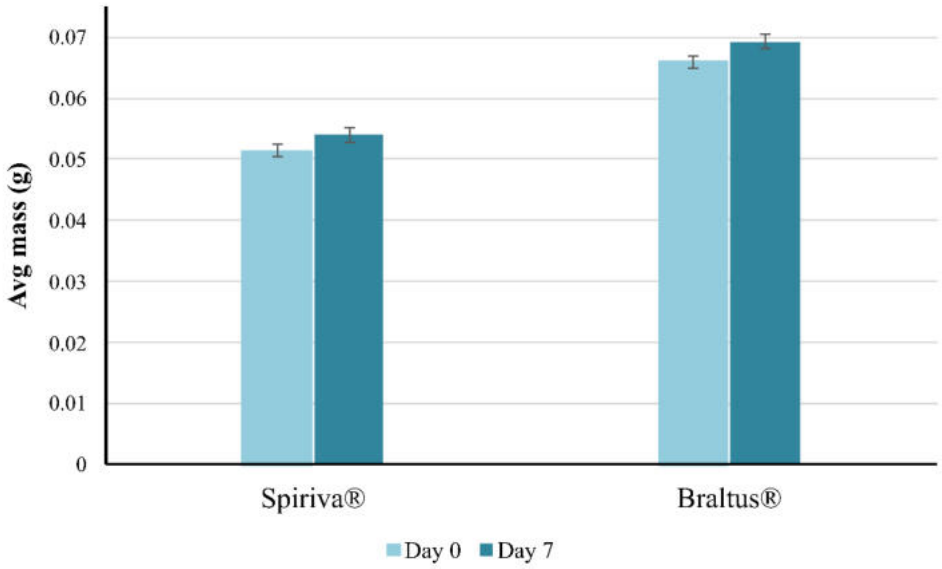

Figure 6. Average mass of DPI capsules upon removal from packaging (day 0) and after 7 days of storage at RT (day 7). Results expressed as mean ± SD (n = 10)

Figure 7 displays the results from the same experiment but conducted under accelerated storage conditions (40 °C and 75% RH). After seven days in the climate chamber, both Spiriva® and Braltus® capsules demonstrated mass increases of approximately 6.9%.

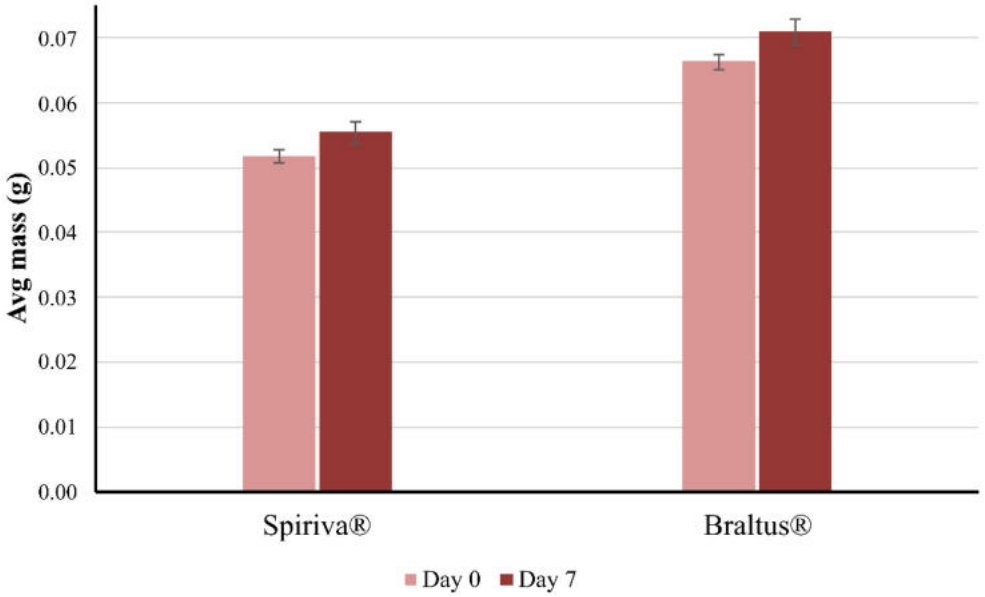

Figure 7. Average mass of DPI capsules upon removal from packaging (day 0) and after 7 days of storage in a climate chamber (day 7) at 40°C, 75% RH. Results expressed as mean ± SD (n = 10)

4.1.4. Karl Fischer moisture analysis

The water content of the powder formulations inside the capsules was measured at three time points: immediately after removal from the original packaging, after 7 days of storage at RT in a pill dispenser, and after 7 days in a stability chamber set to 40 °C and 75% RH. The results are presented in Figure 8.

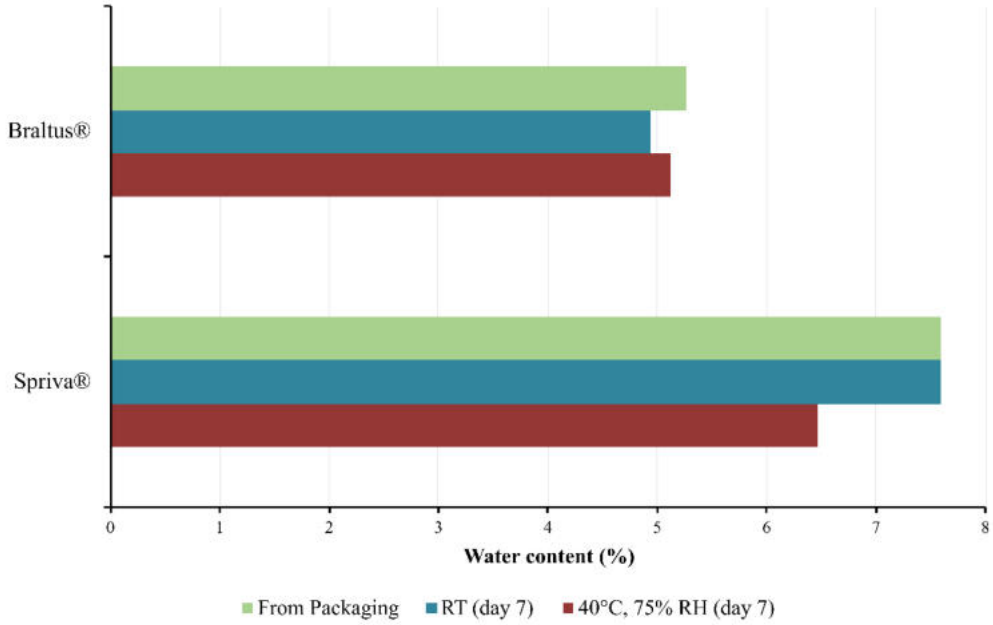

Figure 8. Water content (%) of DPI powder formulations determined by Karl Fischer titration: immediately after packaging removal, following 7 days at RT, and after 7 days in a climate chamber. Results expressed as mean. (n = 3)

For Spiriva®, a minimal increase in water content was observed after storage RT (+0.0132%), while a marked decrease occurred after storage in the climate chamber (−14.8%). In contrast, Braltus® showed a reduction in water content under both conditions, with decreases of 6.1% at RT and 2.8% in the climate chamber.

4.2. Gelatin and HPMC capsule study

4.2.1. Moisture uptake

Both gelatin and HPMC capsules exhibited an increase in mass over time as a result of moisture absorption under the controlled humidity conditions in the climate chamber. Nevertheless, the magnitude and rate of this mass gain differed between the two capsule types (Figure 9).

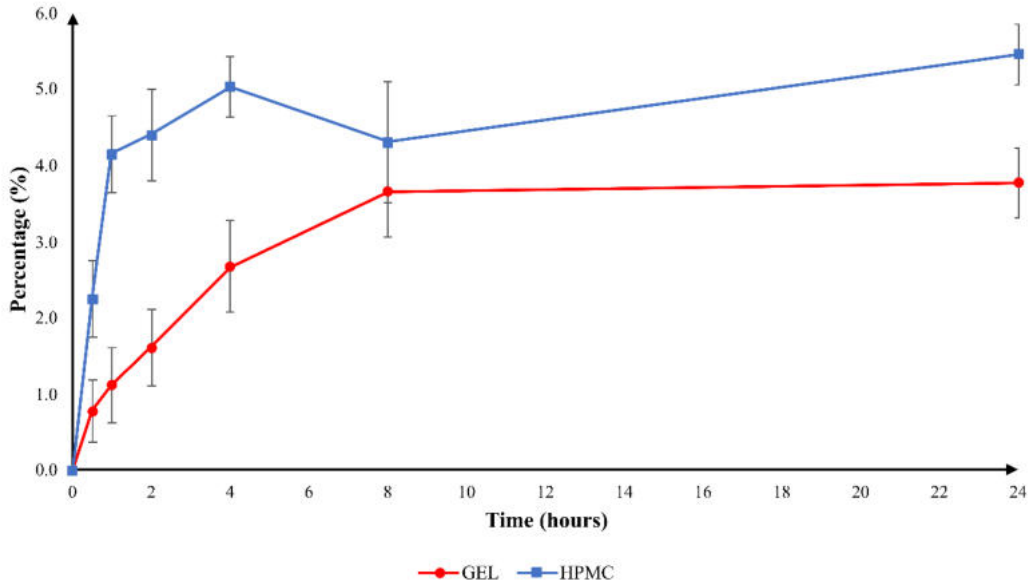

Figure 9. Mass gain (%) of gelatin and HPMC capsules over 24 hours at 25°C and 75% RH. Results expressed as mean ± SD. (n = 20)

As shown in Table 4, HPMC capsules exhibit faster and higher moisture uptake compared to gelatin capsules, especially during the initial hours of exposure. Within the first 30 minutes, both capsules exhibited an increase in mass, with HPMC capsules showing a significantly greater percentage increase between before and after storage (2.25%), compared to gelatin capsules (0.78%). By 1 hour, the mass of HPMC capsules increased by 4.15%, while that of gelatin capsules increased by 1.12%, exhibiting a lower rate of absorption.

Table 4. Moisture uptake and water content of gelatin and HPMC capsules stored at 25 °C, 75% RH over time. Values are presented as the mean ± SD

Time point	Capsule type	Mass gain (%)	Moisture content (%)
0h (dry)	Gelatin	0	10.33 ± 0.41
	HPMC	0	6.27 ± 0.27
30 min	Gelatin	0.78 ± 0.4	11.60 ± 0.37
	HPMC	2.25 ± 0.5	6.37 ± 0.11
1h	Gelatin	1.12 ± 0.5	13.36 ± 0.45
	HPMC	4.15 ± 0.5	8.03 ± 0.74
2h	Gelatin	1.61 ± 0.5	13.00 ± 0.21
	HPMC	4.41 ± 0.6	7.83 ± 0.08

Time point	Capsule type	Mass gain (%)	Moisture content (%)
4h	Gelatin	2.67 ± 0.6	14.82 ± 0.96
	HPMC	5.03 ± 0.4	6.60 ± 0.28
8h	Gelatin	3.66 ± 0.6	11.07 ± 0.69
	HPMC	4.30 ± 0.8	5.53 ± 0.20
24h	Gelatin	3.77 ± 0.5	15.05 ± 0.16
	HPMC	5.46 ± 0.4	8.78 ± 0.40

Over the following hours, HPMC capsules continued to take up moisture, reaching a maximum of 5.46% at 24 hours, although slight fluctuations were observed between the 4 and 8-hour marks. In comparison, gelatin capsules showed a more consistent uptake pattern, gradually increasing and stabilizing at 3.77% by 24 hours, with minimal variation beyond the 8-hour point.

Overall, HPMC capsules absorbed more moisture than gelatin capsules, particularly during the early stages of exposure. The absorption rate for HPMC capsules fluctuated, while gelatin capsules showed a more gradual, steady uptake of moisture throughout the duration of the study.

4.2.2. Visual observations

To illustrate the extent of the moisture uptake under the controlled environmental conditions, images were taken of capsules filled with humidity-sensitive silica beads: Figure 10 shows HPMC capsules containing silica beads following 30 minutes (left) and 24 hours (right) of humidity exposure, capturing the external appearance of the capsules as well as the internal state of the beads, and illustrating the color progression over time.

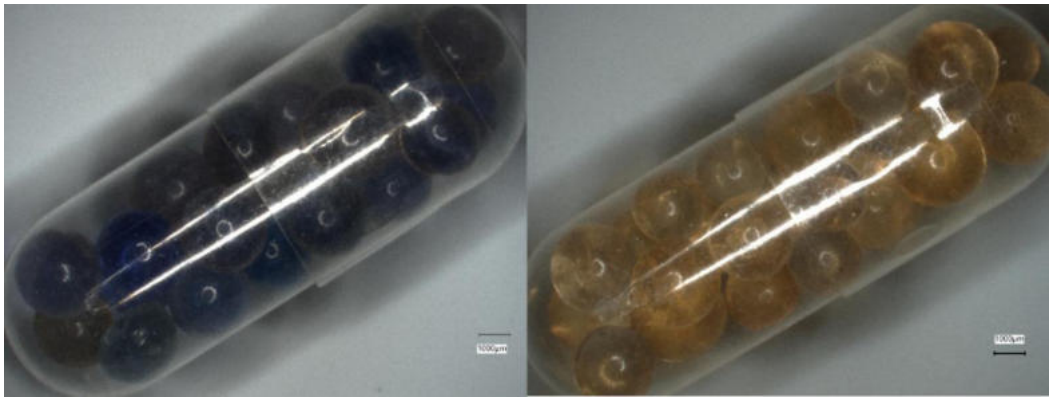

Figure 10. Moisture-induced visual changes in silica beads enclosed in HPMC capsules after 30 min (left) and 24 h (right) under 25 °C and 75% RH

For comparison purposes, reference images were also included: Figure 11 shows silica beads in their fully dry (blue) and fully saturated (brown) states, providing a basis for comparison to evaluate the extent of color change resulting from humidity exposure.

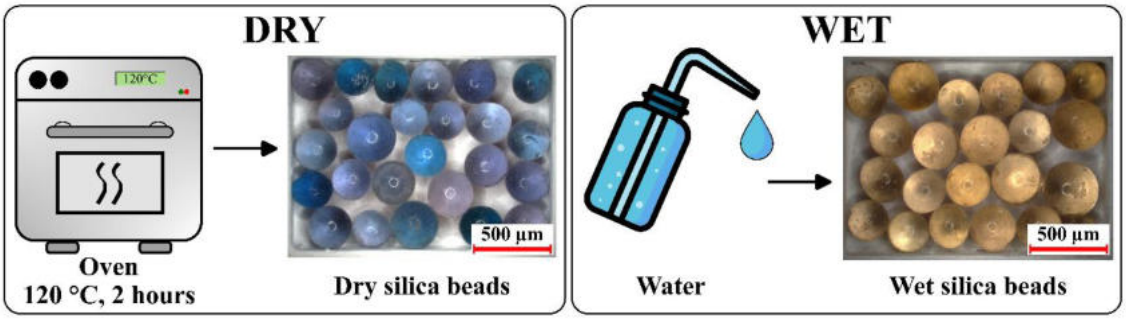

Figure 11. Silica beads in dry and saturated states, serving as visual references for color changes resulting from moisture absorption

Additionally, Figure 12 provides a comprehensive view of the time-dependent visual transformation of the silica beads within both gelatin and HPMC capsules during their exposure to the humid environment.

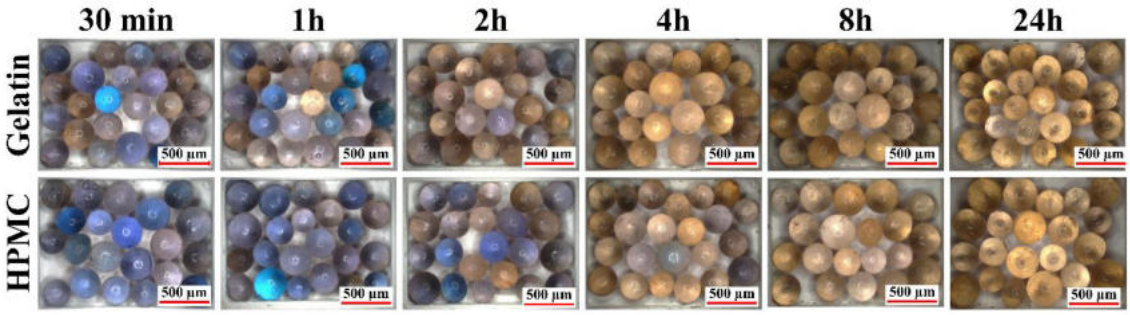

Figure 12. Progression of moisture-induced color changes in silica beads within gelatin and HPMC capsules across multiple time points at 25 °C and 75% RH

At the start of the experiment, both gelatin and HPMC capsules contained completely dry, blue-colored silica beads. However, visual differences between the capsule types were quickly observed: after 30 minutes of exposure, a color shift towards brown was observed in some beads kept in gelatin capsules, indicating an early moisture uptake. In contrast, the beads in HPMC capsules largely retained their blue color, indicating minimal water absorption at this early time point.

At 1 hour, the beads in the gelatin capsules showed a more noticeable color change, with some beads turning brown and an overall decrease in blue tones. In contrast, the beads in HPMC capsules mostly retained their blue tones, although some very light brownish coloration began to appear. The divergence in behavior continued over the 2 and 4-hour marks, with gelatin beads becoming increasingly darker, while HPMC capsules showed a slower and less intense color change.

By 8 and 24 hours, silica beads in both capsule types had predominantly turned brown, though with differing intensities. The beads in the gelatin capsules were visibly darker than those in the HPMC capsules, confirming a greater degree of moisture absorption.

These visual findings underscore the higher sensitivity of gelatin capsules to environmental humidity when compared to HPMC capsules under identical storage conditions.

4.2.3. Hardness

The measured hardness values for gelatin and HPMC capsules at each time point are summarized in Table 5. These results reflect the structural response of the capsule shells to the exposure to humid conditions for different durations.

Table 5. Changes in capsule hardness over time at 25 °C, 75% RH. Values are presented as the mean ± SD

Time point	Capsule type	Hardness (N)
0h (dry)	Gelatin	60 ± 5.6
	HPMC	57 ± 5.4
30 min	Gelatin	-
	HPMC	-
1h	Gelatin	64 ± 3.4
	HPMC	44 ± 0.4
2h	Gelatin	56 ± 4.1
	HPMC	45 ± 0.4
4h	Gelatin	22 ± 1.5
	HPMC	41 ± 2.9
8h	Gelatin	25 ± 1.8
	HPMC	43 ± 4.4
24h	Gelatin	-
	HPMC	-

Empty cells ("-") indicate data not explicitly provided for that time point.

Gelatin capsules demonstrated a marked decline in hardness as the exposure time increased. Notably, a sharp reduction was observed at 4 hours and after, indicating a critical threshold beyond which the capsule structure softened considerably. This pronounced loss in mechanical rigidity is consistent with the known hygroscopic nature of gelatin and suggests rapid plasticization of the capsule wall once sufficient moisture has been absorbed.

In comparison, HPMC capsules displayed a more moderate and gradual decrease in hardness across the same time frame. Rather than undergoing a sudden loss of rigidity,

the HPMC capsules softened progressively, suggesting a slower and more controlled interaction with moisture.

4.2.4. Moisture analysis

To evaluate the extent of moisture uptake during the exposure to the humid conditions, thermal analysis was conducted for gelatin and HPMC capsules. The results, summarized in Table 4 and Figure 13, provide insight into how each capsule type interacted with the environmental humidity over time.

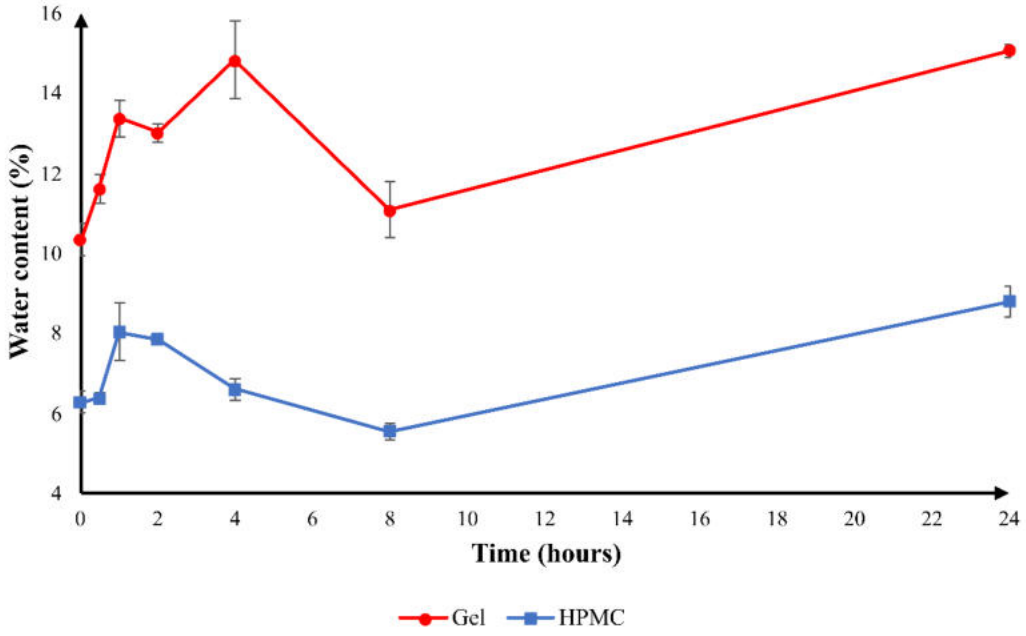

Figure 13. Water content (%) of gelatin HPMC capsules stored under 25°C and 75% RH. Results expressed as mean ± SD (n = 5)

At the initial time point (0 hours), gelatin capsules had an average water content of 10.33%, while HPMC capsules began with a lower value of 6.27%. These baseline differences reflect material-dependent moisture retention prior to environmental exposure. This is consistent with their respective Loss on Drying (LOD) values (14.7% for gelatin and 4% for HPMC) according to the manufacturers' certificates of analysis. After exposure, during the first hour, both capsule types exhibited an increase in moisture content, with gelatin reaching an average of 13.36%, while HPMC capsules increased to 8.03%, indicating early uptake of moisture from the surrounding environment.

Interestingly, moisture uptake did not follow a strictly linear trend. A notable decrease in moisture content was observed in both capsule types between 4 and 8 hours. Gelatin capsules experienced a considerable decrease in water content, dropping from 14.82% to 11.07%, while HPMC capsules, which had already started decreasing from the 1-hour time point, continued to decline, reaching 5.53%.

By 24 hours, both capsule types showed a steady increase in moisture content, suggesting continued interaction with the humid environment. The observed trend indicates that moisture uptake did not follow a strictly linear trajectory but rather appeared to involve dynamic interactions between the capsule materials and the surrounding humidity, which may be attributed to processes such as partial moisture loss, internal redistribution within the capsule shell before reaching a new equilibrium.

4.2.5. Wettability

To evaluate potential changes in surface wettability after 24 hours of exposure to humid conditions, sessile drop tests measurements performed on both gelatin and HPMC capsules. The contact angle, which reflects the extent of surface hydrophilicity, served as an indicator of the interaction between the capsule surfaces and water both prior to and following moisture uptake. Microscopic images of the sessile drops are presented in Figure 14.

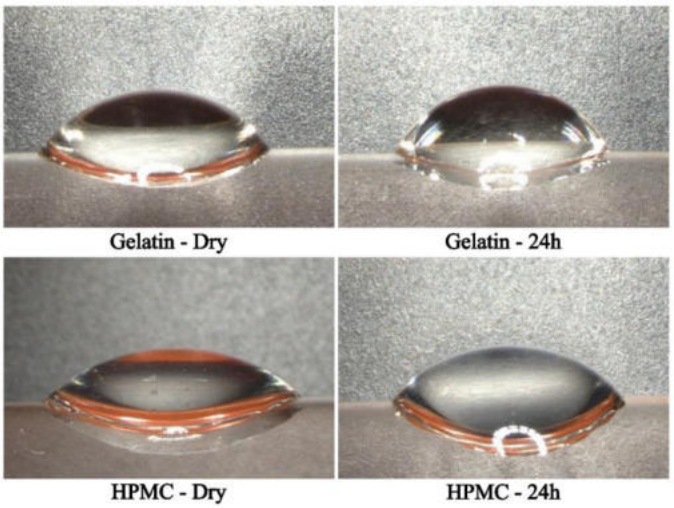

Figure 14. Microscope images of the sessile drops on gelatin and HPMC capsule surfaces before and after 24 hours of storage at 25 °C and 75% RH

In addition, corresponding contact angle values for both capsule types in dry and humidity-exposed states, along with standard deviations (SD) and statistical significance (p-values), are provided in Table 6.

Table 6. Mean contact angle values (°) for gelatin and HPMC capsules in the dry state and after 24 hours of exposure to 25 °C, 75% RH (n = 5), with corresponding p-values. Values are presented as the mean ± SD

Capsule type	Condition	Average contact angle	p-value
Gelatin	Dry	53° ± 4.2°	0.01
	After 24h exposure	60°± 2.7°	
HPMC	Dry	44° ± 3.2°	0.06
	After 24h exposure	49° ± 1.2°	

Contact angles below 90° are typically indicative of hydrophilic surfaces, with values between 30° and 70° commonly associated with moderate wettability. This range suggests that the surface allows for a partial spreading of water without complete wetting. The lower contact angles observed for HPMC capsules point to a relatively higher hydrophilicity, whereas the slightly elevated angles measured for gelatin capsules indicate a surface that is less wettable yet remains hydrophilic.

In the case of gelatin capsules, the mean contact angle increased from 53° (dry state) to 60° (24h of humid conditions). This change was statistically significant ($p = 0.01$), indicating a modest decrease in surface hydrophilicity after moisture uptake. Despite this angle increase, the surface wettability remained within the moderate range: it indicates a shift toward a lower hydrophilicity, but not sufficient to categorize the surface as hydrophobic.

In contrast, HPMC capsules showed an increase in average contact angle from 44° to 49° over the same period. Although an upward trend was observed, it did not reach statistical significance ($p = 0.06$), suggesting that the surface wettability of HPMC capsules was essentially unaffected by the moisture exposure.

Overall, the measured contact angle values confirm that both capsule types remained within the hydrophilic range and maintained their initial wettability characteristics. Moreover, under both conditions, HPMC consistently showed lower contact angles than

gelatin, indicating its slightly higher surface wettability. These results suggest that 24 hours of humidity exposure did not substantially affect surface hydrophilicity, and the minor or non-significant increases in contact angle imply that any observed performance changes are more likely due to internal structural alterations rather than modifications at the capsule surface.

4.2.6. Mechanical analysis

4.2.6.1. Horizontal deformation

To evaluate changes in lateral stiffness and mechanical integrity under humid conditions, horizontal deformation testing was carried out on both gelatin and HPMC capsules. The force–deformation responses (load in grams vs. displacement in millimeters) are depicted in Figure 15 (a) and 15 (b), and the maximum load values at each time point are summarized in Table 7. Further quantitative parameters (stiffness and AUC), are provided in Table 8.

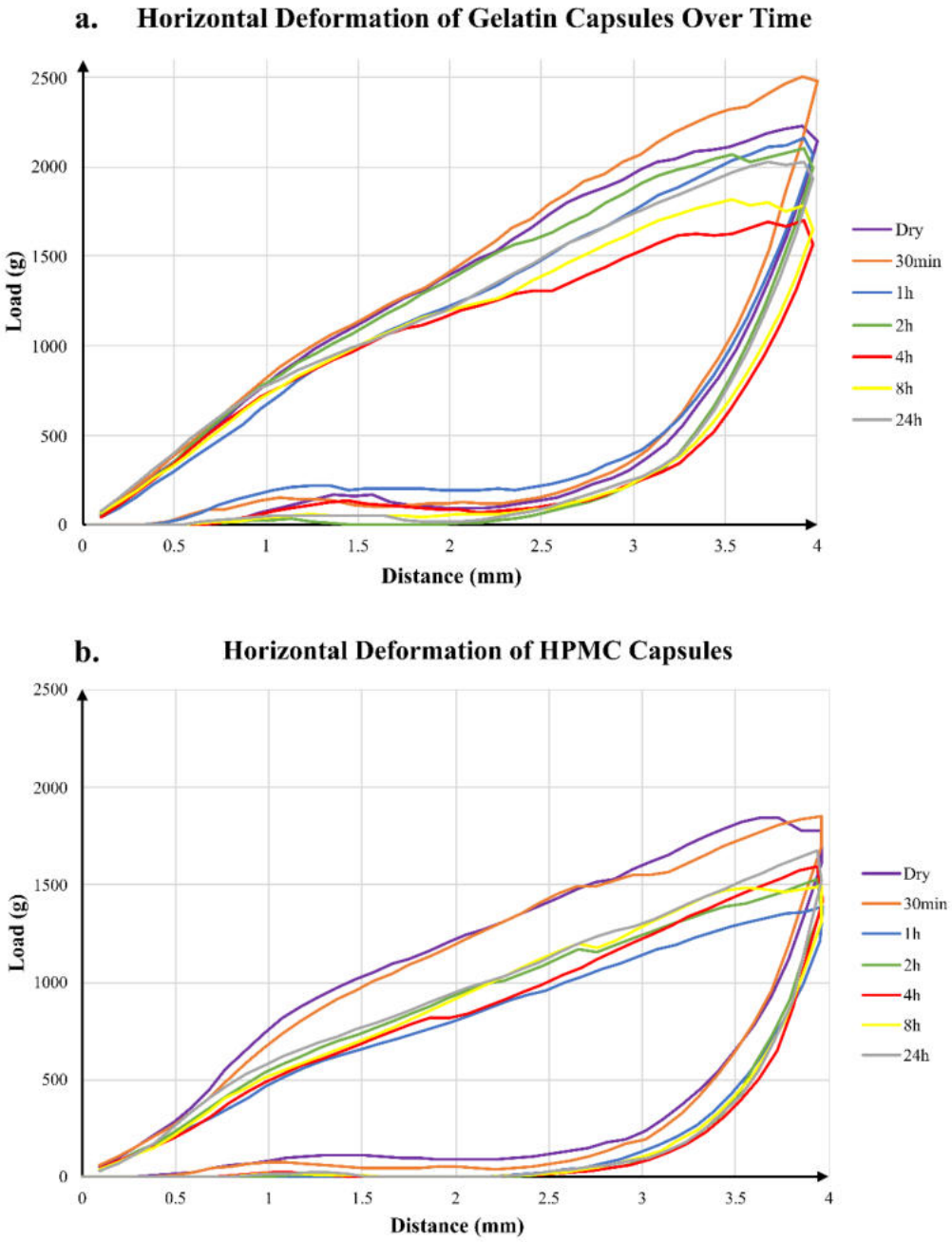

Figure 15. Horizontal deformation profiles of gelatin (**a**) and HPMC (**b**) capsules in the dry state and after exposure to 25 °C and 75% RH at various time points

Table 7. Mechanical resistance of gelatin and HPMC capsules: deformation and puncture behavior at 25 °C, 75% RH over time. Values are presented as the mean ± SD

Time point	Capsule type	Max. horizontal deformation force (g)	Max. vertical deformation force (g)	Max puncture force (g)
0h (dry)	Gelatin	2230.0	2897.4	582.1 ± 59.1
	HPMC	1845.0	2947.3	525.5 ± 89.1
30 min	Gelatin	2506.6	2993.1	519.1 ± 79.1
	HPMC	1846.8	2073.3	465.5 ± 69.7
1h	Gelatin	2157.1	2831.7	383.9 ± 54.0
	HPMC	1385.6	3149.1	496.8 ± 51.9
2h	Gelatin	2103.1	2797.3	534.7 ± 60.0
	HPMC	1531.2	2536.8	388.4 ± 37.6
4h	Gelatin	1701.5	1351.6	555.4 ± 76.0
	HPMC	1592.7	1157.4	423.4 ± 42.1
8h	Gelatin	1817.1	2406.8	555.1 ± 27.5
	HPMC	1490.4	2076.3	493.1 ± 26.8
24h	Gelatin	2029.3	1991.6	506.8 ± 35.6
	HPMC	1676.6	2122.9	411.2 ± 52.0

Table 8. Stiffness and AUC values for gelatin and HPMC capsules in the dry state and following storage at 25 °C and 75% RH for different durations, under horizontal deformation testing. Values are presented as the mean (n = 5) ± SD

		Stiffness (N/mm)		**AUC (N/mm)**	
		Gelatin	HPMC	Gelatin	HPMC
Horizontal deformation	Dry	5.71 ± 0.459	4.63 ± 0.871	37.6 ± 2.0	34.3 ± 3.2
	30 min	6.24 ± 1.320	4.54 ± 1.141	42.8 ± 4.6	33.8 ± 2.7
	1h	5.55 ± 0.144	3.40 ± 0.226	29.8 ± 2.0	25.4 ± 2.9
	2h	5.34 ± 0.499	3.69 ± 0.912	38.7 ± 1.4	28.5 ± 3.0
	4h	4.09 ± 0.305	3.87 ± 0.891	31.4 ± 1.2	27.4 ± 3.8
	8h	4.55 ± 0.511	3.92 ± 0.844	33.5 ± 1.8	35.4 ± 4.1
	24h	4.96 ± 0.635	3.96 ± 0.746	35.4 ± 4.1	30.1 ± 1.9

Initially, both gelatin and HPMC capsules demonstrated relatively high stiffness and mechanical strength when subjected to the horizontal compression. However, upon exposure to the humid conditions, a progressive decline in resistance to deformation was observed in both capsule types, though the changes happened at different rates.

HPMC capsules exhibited a more rapid reduction in stiffness during the early stages of exposure, particularly by 1 and 4 hours. In contrast, gelatin capsules underwent a more gradual weakening over time, accompanied by a partial recovery after 4 hours. HPMC showed a more dynamic mechanical response, characterized by an initial softening, slight increases in strength at 2 and 4 hours, but a continued overall reduction in mechanical resistance.

By the 24-hour timepoint, both capsule types demonstrated partial recovery. Gelatin capsules displayed a more continuous and stable improvement in stiffness, whereas the stiffness of HPMC capsules appeared to stabilize with only minor increases. Across all time points, HPMC required consistently lower forces to achieve horizontal deformation compared with gelatin, indicating a consistently lower mechanical stiffness.

4.2.6.2. Vertical deformation

Vertical deformation tests were conducted to examine changes in axial stiffness and mechanical resistance of gelatin and HPMC capsules under controlled humidity conditions. The force–deformation responses (load in grams vs. displacement in millimeters) are illustrated in Figure 16 (a) and 16 (b), and the maximum load values at each time point are summarized in Table 7. Additional quantitative parameters, (stiffness and AUC), are detailed in Table 9.

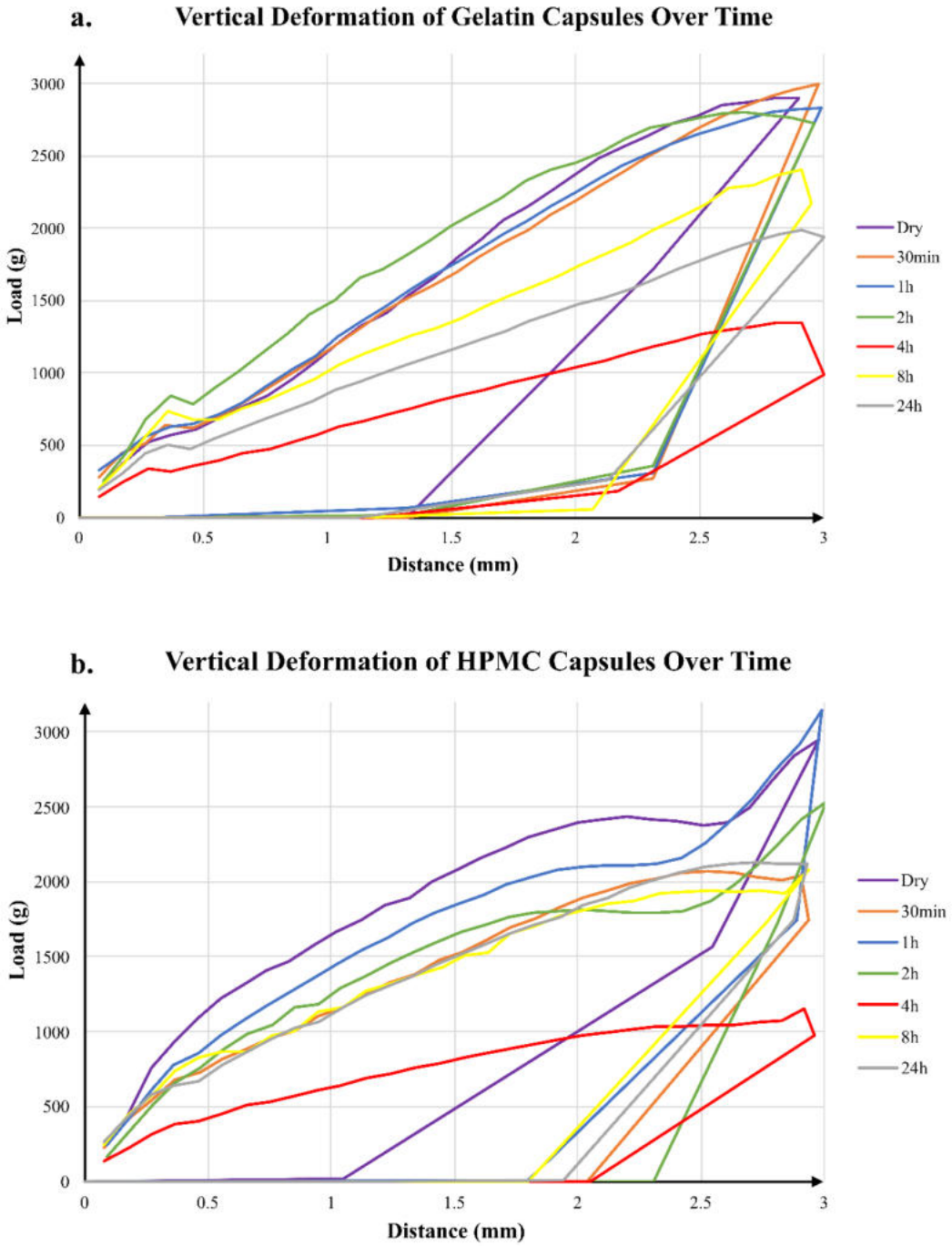

Figure 16. Vertical deformation profiles of gelatin (**a**) and HPMC (**b**) capsules in the dry state and after exposure to 25 °C and 75% RH at various time points

Table 9. Stiffness and AUC values for gelatin and HPMC capsules in the dry state and following storage at 25 °C and 75% RH for different durations, under vertical deformation testing. Values are presented as the mean (n = 5) ± SD

		Stiffness (N/mm)		AUC (N/mm)	
		Gelatin	HPMC	Gelatin	HPMC
Vertical deformation	Dry	10.36 ± 0.614	7.44 ± 0.244	25.8 ± 4.35	23.6 ± 4.09
	30 min	9.57 ± 0.244	6.86 ± 0.377	25.8 ± 2.61	24.9 ± 1.03
	1h	9.40 ± 1.033	7.76 ± 0.437	26.0 ± 2.59	35.1 ± 1.61
	2h	9.52 ± 0.188	6.27 ± 0.320	31.2 ± 1.08	30.1 ± 1.87
	4h	7.55 ± 0.097	5.77 ± 0.318	22.7 ± 2.83	22.5 ± 1.37
	8h	7.22 ± 0.130	5.72 ± 0.280	21.4 ± 2.54	19.7 ± 0.99
	24h	7.44 ± 0.275	7.00 ± 0.826	22.5 ± 2.00	22.8 ± 4.90

Initially, gelatin capsules exhibited greater stiffness and mechanical compared to HPMC capsules. When subjected to humid conditions, HPMC capsules demonstrated an initial increase in stiffness at the 1-hour timepoint, before softening sharply by the 4-hour mark. In contrast, gelatin capsules experienced a more gradual and continuous weakening throughout the exposure period. By the 4-hour timepoint, both gelatin and HPMC capsules reached their lowest vertical mechanical strength. Subsequently, gelatin capsules showed a partial recovery beginning at 8 hours but did not fully regain their original mechanical resistance. Meanwhile, HPMC capsules continued to soften beyond 4 hours progressively but recovered almost to their initial stiffness by 24 hours.

Overall, the vertical deformation results revealed that HPMC capsules have a more dynamic yet reversible mechanical response to moisture exposure, while gelatin capsules, on the other hand, underwent a more progressive and less reversible weakening.

4.2.6.3. Puncture tests

To evaluate the resistance of capsules to mechanical perforation, puncture tests were performed on both gelatin and HPMC capsules. The peak puncture forces measured for gelatin and HPMC capsules are summarized in Table 7 and visualized in Figure 17, which

illustrates the trends across conditions. Figure 18 shows the force-displacement curves for each capsule type, highlighting the point of perforation corresponding to the recorded peak force values.

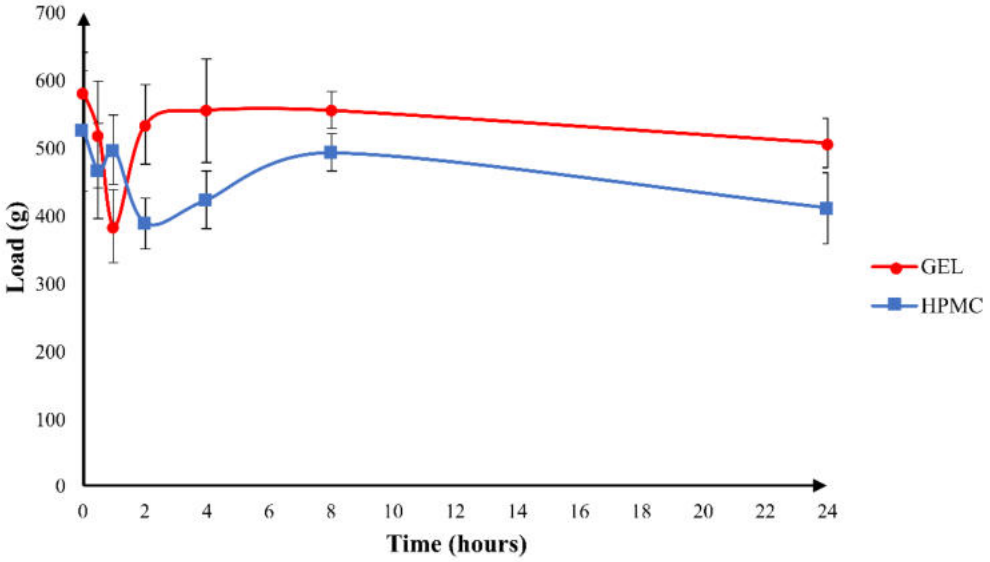

Figure 17. Peak puncture forces of gelatin and HPMC capsules stored at 25°C and 75% RH for up to 24 hours. The puncture force represents the maximum force required to perforate the capsule wall at each time point. Results expressed as mean ± SD (n = 5)

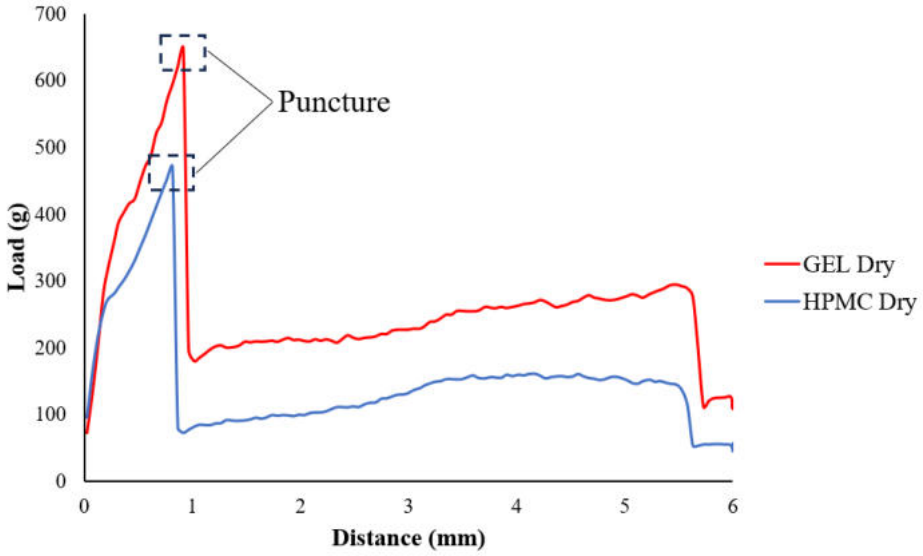

Figure 18. Puncture force profiles of dry gelatin and HPMC capsules, with the peak force marking the point of capsule perforation

The initial puncture testing revealed that dry gelatin capsules required a higher puncture force than HPMC capsules, indicating a higher initial mechanical resistance. However, once exposed to the humid conditions, the puncture resistance of gelatin capsules decreased rapidly, reaching its lowest within the first hour. Following this initial drop, a partial recovery in puncture strength was observed, with values stabilizing from 2 to 8 hours of exposure. After 8 hours, a slight decline was again observed by the 24-hour timepoint.

In contrast, HPMC capsules demonstrated a different pattern. Their puncture force values did not decline immediately but showed a more gradual and variable trend across the earlier time points. Notably, the puncture strength of HPMC capsules seemed to stabilize by 8 hours, followed by a slight decline by 24 hours, similar (but slightly more pronounced) to the trend observed in gelatin capsules.

In summary, while gelatin capsules initially displayed superior puncture resistance compared to HPMC, they were more sensitive to humidity, undergoing a faster and more noticeable reduction in strength. HPMC capsules, although starting with lower puncture resistance, exhibited a more fluctuating yet comparatively stable profile throughout the exposure period.

4.2.7. Positron Annihilation Lifetime Spectroscopy (PALS) analysis

PALS was used to investigate the free volume characteristics of gelatin and HPMC capsules over the 24-hour humidity exposure period. The analysis focused on three key parameters: the o-Ps lifetime, the o-Ps intensity, and the mean lifetime. These parameters, which served as indicators of microstructural behavior at the molecular level in response to moisture uptake, are detailed in Table 10 for all the time points.

Table 10. PALS parameters of gelatin and HPMC capsules during 24h humidity exposure at 25 °c, 75% RH

Time point	Capsule type	o-Ps lifetime[1] (Ps)	o-Ps intensity (%)	Mean lifetime[1] (Ps)
0h (dry)	Gelatin	~1545	~9.8	~377
	HPMC	~1830	~12.9	~ 457
30 min	Gelatin	-	-	-

Time point	Capsule type	o-Ps lifetime[1] (Ps)	o-Ps intensity (%)	Mean lifetime[1] (Ps)
	HPMC	↑	↓	↑
1h	Gelatin	-	-	-
	HPMC	stable	↓	↓
2h	Gelatin	↑	↑	↑
	HPMC	↑	↑	↑
4h	Gelatin	stable	↑	↑
	HPMC	↓	↑	↑
8h	Gelatin	-	-	-
	HPMC	-	-	-
24h	Gelatin	~1600	~11.3	~420
	HPMC	~1925	~11.8	~455

"↑" and "↓" indicate relative trends compared with previous time points.

Empty cells ("-") indicate data not explicitly provided for that time point.

[1] The o-Ps lifetime corresponds to the lifetime of ortho-positronium trapped in free volume holes, while the mean lifetime represents the overall average lifetime of positrons annihilating in all environments within the material (free volume regions and denser areas).

Figure 19 (a) displays the o-Ps lifetime values, which correlate with the size of the free volume holes within the capsule material. HPMC capsules consistently showed significantly higher o-Ps lifetime values than gelatin capsules throughout the measurement period. Initially, HPMC showed a lifetime of approximately 1830 ps, which increased to around 1925 ps by the 24-hour mark, indicating an expansion of free volume. Although the trend showed an overall increase, minor fluctuations were observed at intermediate time points, indicating structural adaptations in response to moisture exposure. These variations suggest a dynamic response which could be associated with moisture-induced plasticization or other microstructural changes in HPMC capsules.

Gelatin capsules, in contrast, displayed a relatively stable o-Ps lifetime profile, with values ranging between approximately 1545 and 1600 ps across all the measured time points: a slight but continuous increase suggesting a gradual and steady structural transformation.

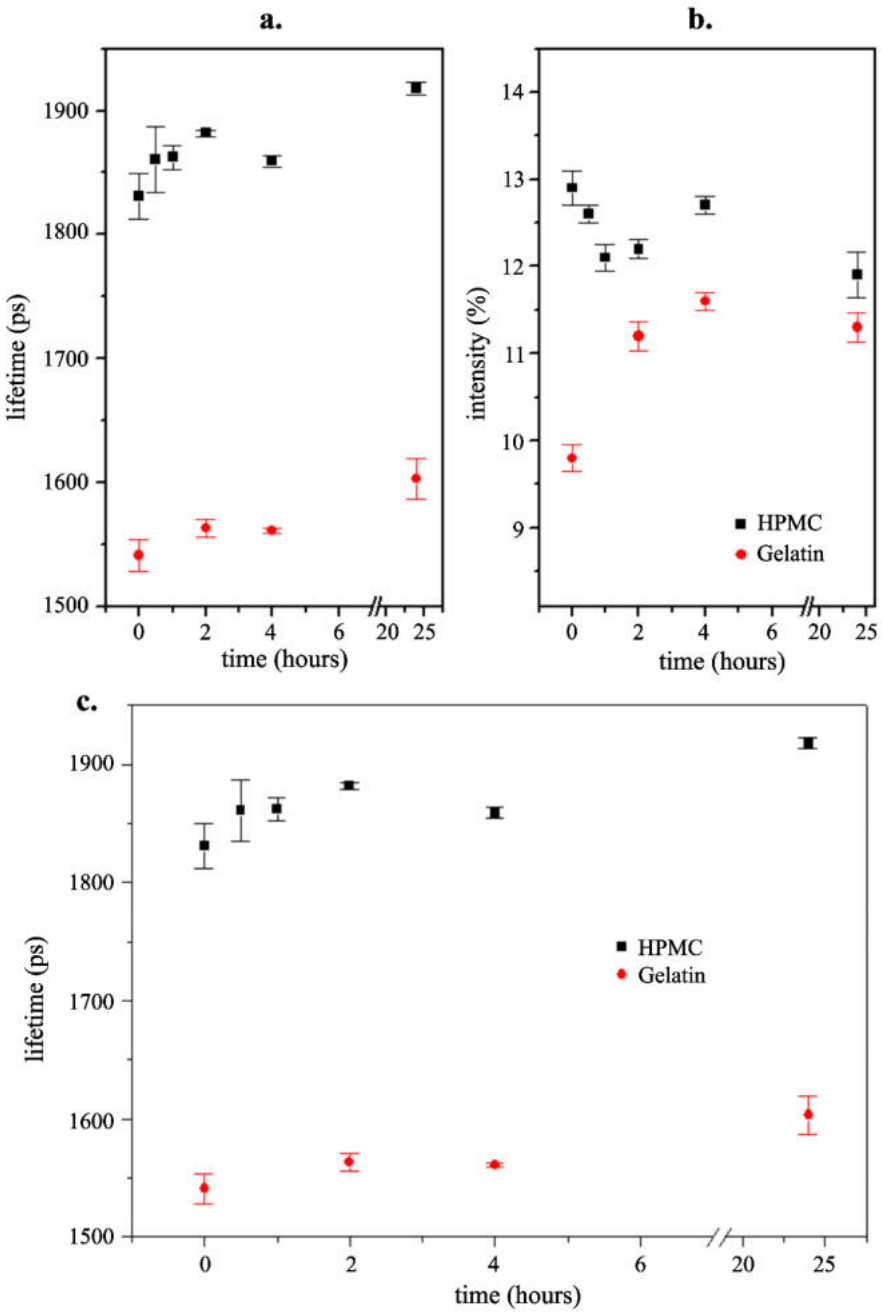

Figure 19. (**a**) o-Ps lifetime, (**b**) o-Ps intensity, and (**c**) mean lifetime measured in gelatin and HPMC capsules stored at 25 °C and 75% RH over a 24-hour period. The o-Ps lifetime corresponds to the size of free volume elements within the material, while o-Ps intensity reflects the concentration of positronium formation sites. The mean lifetime provides an overall assessment of positron annihilation behavior. Error bars represent the SD of replicate measurements at each time point

Figure 19 (b) represents the o-Ps intensity values, which indicate changes in the number of sites within the material where o-Ps formation occurs. At the start of the experiment, HPMC capsules showed higher intensity values (~12.9%) compared to gelatin (~9.8%). Over the storage period, HPMC intensity values displayed fluctuating pattern: initially decreasing, followed by an increase, and then a final decrease at 24 hours.

Gelatin capsules showed a different pattern: the intensity gradually increased up to 4 hours, after which it began to decline. By the 24-hour time point, both capsule types had converged to similar intensity values, approximately 11.3% for gelatin and 11.8% for HPMC.

Figure 19 (c) illustrates the mean lifetime values, which reflect the average annihilation behavior across all positron states and can provide a general overview of changes in the polymer matrix. For HPMC capsules, the values initially fluctuated during the early exposure period, but a steady increase was observed between 1 and 4 hours, reaching values in the range of 460 to 475 ps. However, this was followed by a decrease, resulting in a final value of approximately 455 ps at 24 hours.

On the other hand, gelatin capsules started at a lower mean lifetime (~377 ps) but showed a consistent increase trend throughout the study, ultimately reaching ~420 ps at 24 hours, suggesting a continuous free volume expansion in response to moisture absorption.

Overall, the PALS results revealed clear differences in how the two capsule types respond to moisture exposure at the molecular scale. HPMC capsules showed more pronounced fluctuations in free volume parameters, indicating a more dynamic molecular response to moisture exposure, whereas gelatin capsules underwent more gradual structural changes.

5. Discussion

5.1. Preliminary investigation – Moisture sensitivity of marketed DPI products

The initial evaluation of commercial DPI products (Spiriva® and Braltus®) under varying environmental conditions provided important insights into the moisture sensitivity of the powder formulations and potential interactions with the capsule shells (25).

The visual inspection of the capsules revealed early signs of moisture-induced instability. In the case of the Braltus® (HPMC capsules), visible powder aggregation became progressively more pronounced after storage at RT in a pill dispenser. The extent of aggregation increased to a greater extent following storage under elevated temperature and humidity, indicating that the formulation's moisture sensitivity was intensified under these stress conditions (40 °C, 75% RH). Fluctuations in RH can cause moisture uptake and subsequent drying, triggering cycles of dissolution and recrystallization within the formulation. This physical transformation, consistent with the hygroscopic nature of many DPI formulations, promotes solid bridge formation between particles, ultimately leading to irreversible aggregation (104,105). Although Spiriva® capsules (gelatin capsules) are opaque and did not permit direct visual observation of the powder, noticeable discoloration of the capsule shell after the climate chamber exposure pointed to possible interactions between the capsule material and moisture.

SEM imaging of the Braltus® powder formulation encapsulated in an HPMC capsule provided additional evidence of morphological changes. Over time, the powder shifted from a loose, free-flowing state to a denser, aggregated structure, supporting the interpretation that environmental humidity can initiate cohesion among powder particles. This observation aligns with the known effects of hygroscopicity, which can alter the adhesive and cohesive forces among fine particles (particularly those in the respirable size range of 1-5 μm). Such moisture-induced increased formulation size and particle aggregation can lead to irreversible physical instability, significantly impairing the powder's ability to disperse into respirable particles and thus compromising the efficiency of pulmonary drug delivery (25).

The quantitative mass measurements aligned with the visual and microstructural observations. Both Spiriva® and Braltus® capsules showed a measurable increase in total mass after 7 days of storage at RT, with an even greater increase under the accelerated conditions. This moisture absorption is consistent with the hygroscopic nature of common capsule materials such as gelatin and HPMC, which tend to absorb moisture from their surroundings. The greater mass increase observed under elevated temperature and humidity conditions corresponds with the enhanced moisture diffusion and sorption rates typical of such accelerated storage environments.

When considered alongside the mass measurements, the Karl Fischer results suggest that the observed increases in capsule mass were likely due to moisture uptake by the capsule shells rather than the formulations themselves. In the case of Spiriva®, the significant reduction in formulation water content under accelerated conditions (despite an overall mass gain) indicates that the gelatin capsule may have absorbed moisture while the formulation simultaneously lost water, possibly due to internal moisture redistribution. The slight increase in water content at RT further supports this interpretation. For Braltus®, the decrease in formulation water content under both storage conditions, coupled with the mass gain, implies that the HPMC shell may have also absorbed moisture, though potentially to a lesser extent. These findings point to differences in capsule permeability and interaction with internal contents, with gelatin appearing more prone to rapid and extensive moisture uptake than HPMC.

To summarize, Table 11 shows a concise overview of the findings from the evaluation of the commercial DPI capsules.

Table 11. Summary of moisture-related changes in Spiriva® and Braltus® capsules following 7 days of storage at RT and 40°C/75% RH

Parameter	Spiriva® (Gelatin capsule)	Braltus® (HPMC capsule)	Observations
Visual changes	Powder not visible; capsule shell discoloration after 7 days at 40°C, 75% RH	Progressive powder aggregation at RT, intensified under 40°C, 75% RH	Indicates capsule material (Spiriva®) and powder formulation (Braltus®) moisture sensitivity
SEM morphology	-	Shift from dispersed particles to dense aggregates over 7 days	Aggregation linked to moisture-induced cohesion
Mass changes (% increase)	~5.1% at RT; ~6.9% at 40°C, 75% RH	~5.0% at RT; ~6.9% at 40°C, 75% RH	Both absorb moisture, more so at accelerated conditions
Water content of formulation	Slight ↑ at RT (+0.0132%); significant ↓ at 40°C, 75% RH (-14.8%)	↓ at RT (-6.1%) and at 40°C, 75% RH (-2.8%)	Suggests moisture absorbed mainly by capsule shell; possible internal redistribution

Collectively, these early observations from marketed inhalers highlighted that both gelatin and HPMC capsule-based products are sensitive to environmental moisture, with distinct differences in how the capsule shell and internal formulation responded. The visual, structural, and compositional changes observed under both ambient and accelerated conditions underscored the need for a more systematic investigation. The subsequent experimental study focused on isolating the influence of capsule material by using empty gelatin and HPMC capsules stored under controlled conditions. This

approach enabled a clearer understanding of capsule–moisture interactions and their potential impact on mechanical integrity and inhalation performance.

5.2. Initial Phase – Early stability response to moisture exposure (dry to 30 min)

The initial phase represents the first response of the capsules to the moisture uptake. During this phase, which spans from the capsules in their dry state at 0 hours to the first 30 minutes of exposure to 25°C and 75% RH, the capsules began to absorb moisture, and the first measurable effects on their properties were observed.

At 0 hours, both capsule types were in a dry condition, with their physical integrity and structural rigidity preserved. However, following just 30 minutes of exposure, measurable changes could be observed. One of the earliest indicators of moisture interaction was the increase in capsule mass, confirming moisture uptake in both materials. The HPMC capsules, although with a lower initial moisture content, experienced a greater percentage increase in mass compared to the gelatin capsules, suggesting that HPMC capsules absorb moisture more efficiently than gelatin capsules under high humidity conditions. This could be due to HPMC's higher hydrophilicity (106), which enables it to attract and hold more water molecules compared to gelatin. Additionally, HPMC's polymer structure might allow for greater water uptake through interactions between the polymer chains and moisture in the environment (107).

The visual observations of the silica beads' color change offer an additional perspective. While initially filled with blue (dry) silica beads, signs of moisture absorption became visible after just 30 minutes of exposure, with some beads turning beige/brown. Although both capsule types exhibited bead color changes, the gelatin capsules showed a more pronounced shift, with a greater number of beads turning brown compared to the HPMC capsules. This rapid color shift in the gelatin capsules suggests that moisture reached the beads more quickly, likely due to the more permeable nature of the gelatin matrix. However, it's important to note that despite the stronger visual response in gelatin, the mass measurements showed that HPMC capsules actually

absorbed more moisture within the first 30 minutes. This apparent discrepancy may reflect differing moisture absorption and distribution mechanisms between the capsule types, with HPMC potentially absorbing moisture more efficiently at the surface, while gelatin may have allowed faster internal diffusion. In contrast, the HPMC capsules showed a sharp increase in mass during the first hour, but the color change in the silica beads occurred more gradually, beginning around the 1-hour mark. This delay in browning may be due to the slower penetration of moisture into the capsule interior, consistent with the lower permeability of the HPMC matrix. Overall, these findings suggest distinct water distribution dynamics: HPMC likely absorbs and retains moisture primarily at or near the surface, whereas gelatin enables quicker diffusion deeper into the capsule matrix.

In terms of mechanical properties, the capsules displayed distinct behaviors. The gelatin capsules initially had slightly higher hardness values, indicating that they were less flexible and more resistant to deformation compared to HPMC capsules. This higher resistance is corroborated by the puncture test, where gelatin capsules required a higher peak force to rupture compared to HPMC capsules. However, after 30 minutes, both capsule types showed a reduction in puncture force, which indicates that they both absorbed moisture and softened.

The horizontal deformation measurements provided further evidence of mechanical changes. At the start, both gelatin and HPMC capsules showed minimal deformation, which is an expected, typical result for dry capsules. Nevertheless, after 30 minutes, the gelatin capsules showed a noticeable increase in horizontal deformation load, which aligns with the softening observed in the puncture test. In contrast, HPMC capsules showed a minimal deformation response, suggesting a more gradual mechanical response to moisture absorption.

The vertical deformation results reinforced these trends. Initially, gelatin capsules demonstrated higher stiffness in vertical loading, while HPMC capsules showed greater elasticity. After 30 minutes, a difference in behavior between the two capsule types became apparent, specifically in terms of peak loads. Gelatin capsules maintained their structural rigidity, suggesting minimal water uptake within this short timeframe and highlighting their initial resistance to moisture-induced softening. HPMC capsules,

however, began to show early signs of softening. The notable drop in peak load at 30 minutes suggests that HPMC is more sensitive to moisture uptake, which likely begins to disrupt its polymer structure. The notable drop in peak load at 30 minutes suggests that HPMC is more sensitive to moisture uptake, which likely begins to disrupt its polymer structure.

The results of thermal analysis validated gelatin's higher initial moisture and its greater increase after 30 minutes, in contrast to HPMC's more rapid initial moisture absorption. In gelatin, the elevated water content likely contributed to a lowering of the glass transition temperature (Tg), thus facilitating polymer chain mobility and increased softness. HPMC, on the other hand, absorbed moisture more rapidly within the initial phase, but the structural impact appeared less severe, potentially due to its more moisture-compatible polymer composition.

Further insight into the microstructural effects of the moisture absorption was provided by the PALS analysis. The o-Ps lifetime values were initially lower in gelatin capsules, suggesting that they had less free volume at the start, which is consistent with their higher initial moisture content, rigidity, and resistance to puncture in the dry state. In contrast, HPMC capsules showed higher initial o-Ps lifetime values, suggesting greater initial free volume, which could be attributed to their more hydrophilic nature and capacity for rapid moisture absorption. After 30 minutes of exposure, HPMC's o-Ps lifetime increased further, indicating that absorbed moisture contributed to a rapid expansion in free volume within the polymer matrix. Interestingly, this was not reflected in significant horizontal deformation, suggesting that the moisture uptake had not yet caused notable softening of the capsule wall at this stage. On the other hand, gelatin capsules showed a more progressive increase in o-Ps lifetime but with greater horizontal deformation, suggesting earlier softening occurred, even with lower moisture absorption.

Regarding o-Ps intensity, which reflects the number of available free volume sites for positron annihilation, HPMC capsules showed a higher initial intensity, consistent with their greater free volume. Following 30 minutes of exposure, the intensity decreased slightly, potentially due to absorption-induced polymer structure rearrangements, reducing the number of available sites for o-Ps formation. In gelatin, on the contrary, the

o-Ps intensity appeared to increase gradually, suggesting that new free volume sites were forming as water penetrated and disrupted the polymer structure.

Taken together, the data from this early phase reveal a clear divergence in how gelatin and HPMC capsules respond to brief moisture exposure. Gelatin softened more visibly despite absorbing less moisture, highlighting its greater sensitivity to water-induced mechanical changes. HPMC capsules, although they absorbed more moisture, maintained greater structural stability, reflecting a more controlled and progressive interaction with humidity.

5.3. Intermediate phase – Progressive stability changes during moisture exposure (1h to 4h)

During the intermediate phase of humidity exposure, both gelatin and HPMC capsules continued to absorb moisture, but the patterns and implications of that uptake differed notably. From 1 to 2 hours, the rate of moisture uptake began to slow for HPMC, suggesting an approach toward the saturation threshold of the material's available moisture-binding sites. After this period, the moisture content appeared to plateau, indicating a temporary stabilization. Gelatin capsules, on the other hand, maintained a more continuous and gradual increase in moisture content. Between 2 hours and 4 hours, the moisture uptake for both HPMC and gelatin capsules remained consistently increasing. The initial rapid uptake phase ended for HPMC and was replaced by a steady increase in mass. Gelatin also followed a steady mass increase, following its gradual pattern from the earlier timepoints.

The visual observation of the silica gel beads' color changes supported these findings. HPMC capsules began to show the first noticeable transition of the beads from blue to brown around 1-2 hours. This suggested that the moisture may have initially saturated the capsule surface, causing the absorption rate to slow down. As surface saturation was approached, moisture likely penetrated more gradually towards the interior, resulting in a reduced overall rate of color change. Gelatin capsules, however, exhibited a more consistent and faster transition to brown. By 4 hours, both capsule types showed extensive

browning of the beads, indicating a more significant moisture absorption. However, the browning was more pronounced and widespread in gelatin capsules, further supporting the idea that gelatin allowed moisture to reach the beads more quickly and efficiently.

The hardness measurements revealed that both capsule types softened during this phase, but the patterns differed. HPMC capsules experienced a rapid softening between 1 and 2 hours, followed by a period of relative stabilization. This may reflect an early saturation of surface regions followed by slower diffusion into deeper layers. Gelatin capsules, on the other hand, demonstrated a more linear decline in hardness up to 2 hours, after which a sharp drop occurred between 2 and 4 hours. This sharp decrease aligned well with the timing of the silica bead color transition, suggesting that internal softening progressed more dramatically during this later interval.

In terms of moisture content analysis, HPMC capsules witnessed an initial decrease suggesting that moisture penetration into the capsule may have reached a near-saturation point. However, this was followed by a sharp increase, possibly indicating that the HPMC capsules started absorbing moisture at an accelerated rate, either due to deeper diffusion into the capsule matrix or increased retention of moisture at the surface. In contrast, gelatin capsules continued to lose moisture content despite a consistent gain in mass during this period. These observations suggest that structural alterations within the gelatin matrix may have facilitated moisture loss. As the matrix absorbed moisture, it softened or swelled, allowing some of the absorbed moisture to evaporate or escape from the capsule. While moisture may have been released from the capsule surface, the internal silica beads continued to absorb and retain water, as evidenced by their ongoing color change. These findings suggest that the structural softening or swelling of gelatin may allow for simultaneous absorption and partial loss of water, whereas the silica beads remain unaffected by such mechanical changes and continue absorbing moisture from the internal environment.

In mechanical testing, gelatin capsules showed softening in both horizontal and vertical deformation tests, with the lowest resistance recorded at 4 hours (similarly to HPMC). HPMC capsules, by contrast, displayed diverging trends in deformation:

horizontal tests showed initial softening followed by partial recovery, while vertical tests revealed initial stiffness followed by gradual softening. These findings suggest that moisture penetrated HPMC and gelatin capsules through different mechanisms. For HPMC, moisture appears to first affect the capsule walls, causing them to soften first. Gradually, moisture moved deeper into the capsule structure and eventually reached the domes, which explains the differing mechanical responses seen in horizontal vs vertical deformation tests. In contrast, gelatin capsules seemed to have absorbed moisture more uniformly across the entire capsule. Moisture appeared to diffuse relatively uniformly through the gelatin matrix, resulting in a more evenly distributed weakening of the capsule. Consequently, both the walls and domes exhibited similar reductions in mechanical resistance, resulting in a consistent decrease in load during both horizontal and vertical mechanical tests.

The puncture tests showed that, in the case of gelatin capsules, puncture strength (which had continuously declined from the start) increased significantly between 1 and 2 hours. It then stabilized, with a slight increase, possibly indicating that the gelatin matrix began adapting structurally despite ongoing moisture absorption, perhaps due to more uniform moisture absorption throughout the capsule. In contrast, HPMC puncture strength varied during this phase, possibly reflecting surface evaporation dynamics or internal redistribution of the moisture. Additionally, the changes in puncture strength at the 1-hour mark, together with vertical deformation results, suggest that the walls of HPMC capsules were affected by moisture earlier than the domes.

The PALS measurements demonstrated that the o-Ps lifetime in gelatin capsules remained relatively stable, indicating minimal variation in free volume. This consistency is in line with the observed mechanical behavior, gradual softening and steady mass increase. HPMC capsules, however, displayed a more dynamic PALS profile. The o-Ps lifetime gradually increased from the beginning to around 2 hours, which is indicative of moisture-induced expansion of free volume. Nevertheless, after 2 hours, this trend reversed, with a decline in o-Ps lifetime, indicating deeper moisture penetration and a slowdown in absorption, possibly reflecting internal structural rearrangements as the capsule approached a more stabilized state.

In terms of o-Ps intensity, for gelatin, a steady increase was observed from the start, in line with the observed softening and mass gain during this interval, suggesting a continual rise in the number of available free volume sites as the capsule softened and became more hydrated. In contrast, HPMC capsules showed an early decline in intensity, potentially caused by surface moisture loss or redistribution, followed by an increase between 1 and 4 hours, indicating more uniform moisture uptake and greater interaction with the capsule matrix as moisture penetrated deeper into the walls.

Lastly, the mean o-Ps lifetime for gelatin capsules demonstrated a steady increase, aligning with the observed mechanical softening. Meanwhile, HPMC capsules showed an initial decline, likely due to early moisture uptake, followed by an increase as the structure stabilized.

In conclusion, the intermediate exposure period from 1 to 4 hours revealed clear differences in how gelatin and HPMC capsules respond to moisture. Gelatin capsules absorbed moisture steadily, accompanied by gradual softening, whereas HPMC capsules underwent a rapid initial moisture uptake followed by signs of structural stabilization. While both capsule types exhibited changes in molecular structure, as indicated by the PALS analysis, the gelatin capsules experienced more consistent structural softening, whereas HPMC capsules displayed more dynamic changes in free volume.

5.4. Final phase – Late-stage response to moisture exposure (8 h to 24 h)

The final phase of the study revealed the long-term effects of moisture exposure, with clearer distinctions emerging in the behavior and response of gelatin and HPMC capsules. By this stage, both capsule types had absorbed a considerable amount of moisture; however, their structural and physicochemical responses diverged, providing important insights into their stability and suitability for DPI applications under prolonged humid conditions.

The mass measurements showed that gelatin capsules continued to gain mass steadily up to 8 hours, after which the increase leveled off and seemed to reach a plateau, possibly indicating that the capsules approached a saturation point. HPMC capsules, on the other

hand, showed a temporary decrease in mass between 4 and 8 hours, possibly due to the redistribution of moisture at the surface level. HPMC capsules are known to exhibit dynamic moisture behavior. Studies using low-field magnetic resonance imaging (LF-MRI) have demonstrated that moisture within HPMC shells can migrate from the interior toward the surface, facilitating ongoing interactions with the external environment (92). While this internal redistribution does not, in principle, alter the total mass of the capsule (since no moisture is gained or lost during internal movement), it can influence how the capsule interacts with its environment. This internal movement of water occurs within the polymer matrix and, by itself, does not involve an exchange of moisture with the external environment. However, under experimental conditions such as those used in this study (25°C, 75% RH), the surface redistribution may facilitate moisture exchange with the surrounding atmosphere. For instance, if moisture migrates toward the capsule surface, it may become more susceptible to either evaporation or further absorption depending on the surrounding humidity conditions. Therefore, the mass fluctuations observed (particularly in HPMC capsules between 4 and 8 hours) may reflect not only internal moisture redistribution, but also the result of dynamic interactions between the redistributed moisture and the environment surrounding the capsule. Additionally, compared to gelatin capsules, HPMC capsules tend to absorb and retain less moisture, which may lead to more noticeable moisture loss or redistribution under specific conditions (108). This characteristic further supports the observation of temporary mass decreases due to surface-level moisture changes. Therefore, the mass reduction in HPMC capsules during the 4 hours to 8 hours period is likely a result of moisture migrating or evaporating into the environment, reflecting the dynamic moisture redistribution behavior inherent to HPMC materials.

These differences in moisture dynamics were also reflected in the behavior of the silica beads. In gelatin capsules, the beads had fully darkened by 8 hours and maintained that state through 24 hours, indicating a consistent and sustained moisture uptake. HPMC capsules, on the other hand, showed a slower, more gradual blue-to-brown darkening of the beads, consistent with the more fluctuating mass increase and possible redistribution of moisture during this phase. This difference can be attributed to the distinct moisture absorption characteristics of the two capsule types. Gelatin is characterized by a stronger

affinity for moisture and a different water absorption mechanism compared to HPMC: according to Braham et al., gelatin capsules attract moisture more readily and absorb water differently than HPMC capsules. The study also noted that increasing the RH from 50% to 70% induced crystallization of spray-dried lactose within gelatin capsules, highlighting the impact of moisture interactions (109).

In terms of hardness, by 8 hours, the gelatin capsules had softened considerably, likely due to moisture absorption and the resulting structural alterations within the gelatin matrix. This softening can be explained by a reduction in the Tg, which facilitates molecular mobility within the polymer chains and a transition from a rigid to a more rubber-like state. Such behavior is also characteristic of other hydrophilic polymers such as Eudragit, where the presence of moisture or plasticizers reduces intermolecular interactions between polymer chains, enhancing their flexibility and capacity for deformation (110). A comparable effect was reported by Chiwele et al., who observed that gelatin capsules became noticeably softer and sticky after 24 hours of storage at 37 °C and 75% RH (tropical humid conditions), demonstrating the significant impact of humidity on their structural integrity (111). Similarly, Pinto et al. also demonstrated that gelatin capsules show increased tribo-charging after storage at low RH, likely due to moisture loss leading to reduced plasticity and enhanced surface interactions (112). In contrast, unlike gelatin, HPMC capsules softened comparatively less. HPMC capsules were likewise reported by Chiwele et al. to exhibit enhanced structural stability in the presence of moisture, possibly because of their lower hygroscopicity and stronger cohesion between polymer chains (111).

The moisture content results further corroborated the observed moisture behavior of both capsule types: each showed a reduction in moisture between 4 and 8 hours (with gelatin showing a notably more pronounced reduction). In the case of HPMC, this decrease aligned with the observed mass reduction, suggesting surface-level moisture redistribution and slight moisture loss. For gelatin capsules, by contrast, the mass kept rising during this interval, indicating a sustained moisture uptake from the environment despite the observed drop in moisture content. This is consistent with observations that gelatin capsules absorb and retain greater quantities of moisture across various RH levels

compared to HPMC capsules (109). Furthermore, findings indicate that HPMC capsules absorb and retain less moisture than gelatin ones, which aligns with the observed fluctuations in HPMC's moisture uptake behavior (108). Between 8 and 24 hours, a similar increase in moisture content was observed in both capsule types, indicating that the systems had reached a new equilibrium phase characterized by a much slower but ongoing moisture uptake. While the moisture content increase followed a similar trend in both capsule types, the amount of moisture retained by each remained distinct, highlighting the differences in their absorption behavior noted earlier. This indicates that while equilibrium may be reached over time, the retention capacity and underlying absorption mechanisms remain fundamentally different.

The surface wettability results, measured using the sessile drop method, offered additional understanding of how prolonged humidity exposure altered the hydrophilicity and surface properties of gelatin and HPMC capsules, highlighting the impact of moisture on their behavior. Contact angle values, which reflect surface wettability, showed significant differences between the two capsule types after 24 hours at 25°C and 75% RH. Gelatin capsules showed a significant increase in contact angle, indicating a decrease in surface wettability and a shift toward a more hydrophobic behavior. This could be attributed to the development of a more hydrophobic surface layer or alterations in the capsule's surface morphology resulting from moisture-induced plasticization. These modifications in surface properties may lower the capsule's surface energy, as moisture exposure can cause polymer chains to rearrange, reducing the exposure of hydrophilic groups at the surface and thus making the capsule more hydrophobic. This behavior has been reported in numerous polymer systems, where hydration triggers the reorganization of surface segments, resulting in changes in wettability. For instance, a study on poly(dimethylsiloxane) materials demonstrated that upon being in contact with water, surface-dominating methyl groups tilt more toward the surface, indicating a restructuring which affects surface properties (113). Additionally, a study using the sessile drop method on polymer films found that moisture exposure caused surface reorganization, increasing the contact angle and reducing surface energy, which (although conducted on a different polymer system) supports the same mechanism observed in the gelatin capsules (114). On the other hand, HPMC capsules showed a less pronounced, statistically non-

significant increase in contact angle, indicating that the surface wettability remained relatively stable under the same exposure conditions. This behavior is consistent with HPMC's lower moisture sorption capabilities, which contribute to a more stable surface interaction with water molecules. Unlike gelatin, which exhibited a more substantial increase in hydrophobicity, HPMC capsules maintained their overall hydrophilic nature even after prolonged exposure to humidity. This stability could be attributed to the stronger molecular interactions between HPMC polymer chains, which may prevent the migration of moisture to the surface and thereby minimize the changes in surface energy.

In the horizontal deformation tests, the two capsule types exhibited distinct behaviors: for gelatin, the maximum load required to deform the capsule walls initially decreased with moisture uptake, aligning with the observed softening and mass increase. However, from 4 to 24 hours, a progressive increase in maximum load, stiffness, and AUC was observed. This suggests that while initial water absorption led to softening, the gelatin matrix later underwent a form of structural consolidation, making the lateral walls more resistant to deformation. This delayed stiffening effect may reflect a moisture-induced reorganization within the gelatin polymer network. With increasing moisture uptake, the material likely approached saturation, prompting the reformation of intermolecular interactions (such as hydrogen bonds) between polymer chains. This reorganization may have resulted in a denser or more structured matrix, therefore limiting further deformation. A comparable behavior was observed in gelatin capsules stored at 11%, 22%, and 51% RH, where early-stage softening due to moisture absorption was followed by stabilization, potentially connected to structural reorganization (112). Notably though, the maximum load at 24 hours remained lower than the values measured at earlier time points (dry to 2 hours), indicating that the gelatin structure never fully regained its initial mechanical strength. In contrast, HPMC capsules showed a more controlled and progressive mechanical response, with slight softening then stabilization: the stiffness values, in particular, appeared to approach a plateau, further supporting the idea that HPMC maintained a relatively stable lateral wall structure throughout the later phase of moisture exposure. This is consistent with previous findings on HPMC capsules exposed to various humidity levels (11%, 22%, and 51%), which showed only mild

variations in electrostatic response, further confirming the structural stability of HPMC in humid conditions (112).

Further distinctions were observed in the results of the vertical deformation tests, which targeted the capsules domes. During this phase, the gelatin capsules initially stiffened, then underwent softening, which implies that the domes initially resisted compression more (potentially due to localized thickening or structural stiffening) before becoming more flexible again, possibly because of a cumulative plasticization. This behavior indicates an uneven response within the capsule, where the lateral walls became more rigid while the dome area may have gradually softened due to moisture exposure. In contrast, HPMC capsules demonstrated a gradual strengthening of the capsule dome as the material responded to moisture over time. The relatively small variations, aligning with the stable surface wettability, underscore the greater structural stability of HPMC capsules when subjected to moisture stress.

In terms of puncture force, the gelatin capsules showed relatively stable values over time. This response suggests that while softening and mass gain occurred earlier during the exposure, the matrix underwent a degree of structural reorganization that helped preserve puncture resistance. The slight decrease by 24 hours, despite the observed lateral structural stiffening (from the horizontal deformation), may reflect differential responses across the capsule, with the dome area becoming more susceptible to moisture-induced plasticization, consistent with the load decrease observed in the vertical deformation test. HPMC capsules, however, displayed an increase followed by a decrease in puncture force over this period. The intermediate rise in resistance correlates with the vertical deformation, whereas the subsequent decrease may indicate the capsule's adaptation to extended moisture exposure, involving a slight relaxation of the polymer network as it approaches equilibrium. These findings align with the stable contact angle measurements and mechanical stiffness, supporting that HPMC exhibits a dynamic yet generally stable structural behavior under humid conditions. Remarkably, this stability persisted even at lower humidity levels (11% and 33% RH), with HPMC capsules showing less change in puncture properties compared to gelatin capsules (115).

Lastly, with regards to the PALS results, gelatin capsules' o-Ps lifetime progressively increased over time, with a considerable increase at 24 hours, suggesting enhanced

molecular rearrangement and free volume expansion due to plasticization. This increase likely stems from the moisture-induced separation of polymer chains, which creates additional nanoscopic pockets that can trap positrons, therefore increasing the o-Ps lifetime. This behavior is characteristic of hydrophilic polymers undergoing plasticization, as water molecules disrupt intermolecular interactions (particularly hydrogen bonding) resulting in an increased chain mobility and expansion of free volume.

For HPMC capsules, the o-Ps lifetime continued to increase until 24 hours, likely reflecting slow, moisture-driven alterations within the HPMC polymer matrix, similar to the moisture-induced reorganization observed in the horizontal deformation tests, where an increase in load was noted. This response aligns with HPMC's lower moisture affinity and its more consistent structural behavior under humid conditions, as noted earlier. Notably, the o-Ps intensity in HPMC capsules declined at 24 hours, converging with the values observed for gelatin capsules at the same time point. This decline implies that, although the HPMC matrix likely experienced less extensive plasticization than gelatin, extended exposure to moisture may have still induced subtle microstructural changes, promoting the development of free-volume pockets, although to a lesser degree. The observed intensity decrease for both capsule types may indicate a dynamic equilibrium between the ongoing moisture uptake and the polymer chain reorganization within the matrix.

Both capsule types showed an overall increase in free volume as the exposure time progressed; however, gelatin capsules exhibited a more significant degree of molecular mobility, which corresponds with their higher moisture retention and the resulting softer, more plasticized structure. By contrast, HPMC capsules displayed a slower, more controlled molecular rearrangements, reflecting their lower moisture absorption and comparatively stable mechanical properties.

The mean lifetime values further emphasize the differences in molecular dynamics between the two capsule types. HPMC capsules showed initial fluctuations with an increase followed by a decrease and then a rise again at 24 hours, reflecting dynamic but controlled adjustments in free volume consistent with their lower moisture affinity and structural stability. Conversely, gelatin capsules exhibited a steady, continuous rise in mean lifetime throughout the exposure period, indicative of ongoing free volume expansion and sustained molecular plasticization. These patterns reinforce the notion that

gelatin capsules experience more pronounced moisture-induced molecular mobility, while HPMC capsules maintain a more stable polymer network under humid conditions.

5.5. Capsule characterization approach and its significance for DPI systems

The aim of this study was to observe the moisture-induced responses of gelatin and HPMC capsules through a comprehensive, multi-faceted approach. Through a combination of physical, mechanical, and molecular-level assessments, the study evaluated capsule robustness under storage conditions reflective of real-world settings, highlighting implications for DPI performance. A comprehensive understanding of how capsule properties interact with formulation characteristics, device design, and patient-specific variables and usage patterns is crucial to the successful development of DPI products. These interdependencies significantly affect manufacturing consistency, drug delivery efficiency, and overall product performance. These insights are essential to optimizing both formulation and device to ensure a reliable pulmonary drug delivery (77).

Gravimetric measurements were used as a primary quantitative method to assess moisture uptake by capsules. By recording the mass of individual capsules before and after exposure to humidity, the extent of moisture absorption over time was determined, along with its potential effects on capsule integrity and formulation protection. Because moisture uptake can negatively impact both capsule structure and drug stability, changes in mass offer a simple yet effective metric to evaluate DPI suitability (5).

In addition to mass gain, the study employed a novel visual method to qualitatively evaluate the capsules' ability to protect their contents from internal moisture. The color changes of the silica beads provided a clear, qualitative visual indicator of ambient humidity protection, an important aspect for maintaining powder stability and consistent dosing in DPI formulations.

Furthermore, hardness testing was performed to evaluate changes in capsule rigidity, a key factor influencing mechanical performance in DPI applications. Although tablet hardness testers are primarily designed and standardized for measuring the mechanical strength of compressed tablets, their application can be extended to hard capsules in adapted research setups. In this study, the instrument was used to assess the compressive

resistance of gelatin and HPMC capsules as a means of evaluating shell integrity under mechanical stress. This approach is particularly relevant in the context of DPI capsules, where changes in shell strength due to moisture uptake can influence performance parameters such as needle perforation and powder release. As capsules absorb moisture, they may soften, leading to a reduced structural integrity. This weakening is particularly problematic in DPI devices, where capsules must remain sufficiently rigid to be pierced by needles without deforming or collapsing. By evaluating hardness, the study aimed to detect early signs of moisture-induced softening, offering insight into the resilience of each capsule type under humid conditions and its ability to maintain functional performance during drug delivery.

Moisture content analysis was used to assess water uptake at a more material-specific and internal level, providing insight into how moisture is retained within the capsule matrix. This method, which quantifies the actual water content retained in the capsule shell, complements the gravimetric data by offering a more detailed understanding of the extent and potential impact of internal moisture uptake. Elevated internal moisture not only affects mechanical properties like stiffness and brittleness but can also increase stickiness, leading to improper piercing or impaired powder dispersal. These findings complemented the gravimetric data by providing a more elaborate understanding of internal moisture absorption. This is especially important because the varying sorption characteristics of capsule materials like gelatin and HPMC can substantially affect their capacity to maintain formulation stability in humid environments (109).

Contact angle measurements using the sessile drop method were conducted to evaluate changes in capsule surface wettability following humidity exposure. Since wettability reflects how easily liquids (or powders with moisture) interact with the capsule surface, it plays a key role in DPIs by affecting the adhesion between the capsule and the powder, which in turn influences the efficiency of dose release during inhalation. A higher contact angle corresponds to reduced surface hydrophilicity, which may weaken interactions with hygroscopic formulations, potentially facilitating powder release. Oppositely, increased surface hydrophilicity could promote adhesion of powders to the capsule wall, thereby reducing the emitted dose (116,117). Practically speaking, even if changes in contact angle are not statistically significant (as seen with HPMC capsules) moisture uptake can still plasticize and compromise the strength of the capsule wall.

Although the increase in contact angle for HPMC capsules was less significant, the moisture absorbed could still alter their internal structure and influence their ability to be effectively punctured. Since DPI capsules must be reliably pierced by the inhaler device to guarantee a proper aerosolization, any softening caused by moisture could reduce puncture performance and eventually decrease the delivered dose.

In addition, mechanical testing was performed to assess whether moisture exposure led to softening or weakening of the capsule shell, using texture analyzer methods designed to simulate real-world handling and interactions within the inhaler device. The tests were designed to focus on a particular aspect of mechanical performance: horizontal deformation evaluated lateral wall flexibility, vertical deformation targeted on the dome region that is typically pierced in inhaler devices, and puncture testing directly replicated the needle insertion process (103,118). Collectively, these tests provided insights into both localized and general mechanical behavior, allowing for a more comprehensive assessment of how capsule softening could affect DPI performance.

Lastly, to investigate changes at the molecular level, PALS measurements were conducted. To analyze the PALS findings, it is essential to begin with the notion of polymer free volume. Free volume refers to the internal pockets or voids within a polymer structure, which are spaces not occupied by polymer chains or other molecular components (119). Originally conceptualized by Cohen and Turnbull in 1961, it represents the portion of a polymer's thermal expansion that can occur without the input of additional energy (120). These voids arise from inefficient molecular packing, especially within the amorphous regions of a polymer, where disordered arrangements lead to structural gaps (121). In pharmaceutical polymers, free volume is a key factor determined by their molecular organization, influencing how polymers interact with small molecules such as water, and thereby directly affecting moisture uptake. Additionally, variations in free volume impact mechanical stability: polymers with greater free volume typically show increased flexibility or plasticization, whereas reduced free volume is associated with increased brittleness and stiffness.

Gelatin, a protein-based polymer with a semi-crystalline structure, exhibits distinctive free volume characteristics due to its polypeptide chains and complex secondary structures, such as helices and random coils, which together form a structurally

heterogeneous matrix (122). This complex structure leads to a non-uniform free volume distribution, creating different sites for water binding and molecular movement. Because gelatin contains abundant hydrophilic amino acid residues, moisture uptake strongly affects its free volume by allowing water molecules to bind within the polymer network and integrate into its structure (122). These interactions cause notable changes in the distribution of free volume and influence the material's mechanical behavior, as evidenced by PALS results showing a steady increase in o-Ps lifetime with increased humidity exposure.

HPMC, in contrast, has a distinct polymer structure: it is a cellulose derivative composed of glucose units substituted with hydroxypropyl and methyl groups, which notably influence its hydrophilicity and free volume characteristics (123). This results in a more uniform and amorphous polymer structure, where free volume is evenly distributed compared to gelatin. Under moisture exposure, HPMC demonstrates dynamic changes in free volume, with PALS data showing variable o-Ps lifetimes indicative of molecular rearrangements and structural adjustments. This suggests that HPMC accommodates moisture through structural reorganization of its polymer matrix rather than just occupying pre-existing voids.

PALS served as a highly sensitive technique offering molecular-level insights by tracking changes in the free volume within the capsule materials. A reduction in free volume indicates tighter molecular packing, which can lead to increased brittleness or decreased flexibility of the capsule. Unlike mechanical or physical tests that assess macroscopic properties, PALS revealed how moisture influences the capsules' internal structure on a deeper level. By analyzing variations in the energy of annihilation photons, PALS examines the local electron momentum distribution, providing complementary information about the material's molecular and microstructural changes (124). Specifically, PALS detected free volume changes linked to moisture uptake, revealing molecular modifications that traditional mechanical tests might miss. These findings are especially important for polymer systems, where moisture can alter the material's physical properties and stability (125), ultimately impacting the ease with which a DPI capsule can be pierced and its powder delivered.

The fundamental distinctions between gelatin and HPMC capsules are well recognized; however, this study offers several novel contributions that broaden the

evaluation of capsules under humidity stress. In particular, applying PALS to monitor free volume changes in gelatin and HPMC capsules provides a unique and insightful perspective. Although PALS has been previously used in pharmaceutical research to examine microstructures and track changes in various drug delivery systems (125), its targeted application for assessing capsule humidity response represents a new development. Additionally, the incorporation of cobalt(II) chloride-based silica gel beads as indicators of moisture penetration offers a practical and informative technique that has not been previously applied in this context. While these beads are commonly used as desiccants, their use inside capsules to visually track humidity uptake constitutes a novel approach. Similarly, while contact angle analysis is a standard method for evaluating the wettability of pharmaceutical powders, its extension to capsule materials (particularly to assess changes induced by humidity exposure) has not been previously documented. The integrated use of these diverse analytical techniques allowed for a comprehensive correlation between macroscopic and molecular behaviors, enhancing our understanding of how each capsule type performs in humid conditions. This perspective is highly relevant for DPI formulation development and product optimization, where even minor material changes can significantly impact clinical performance, stability, and manufacturing consistency.

Overall, the combined findings of this study (summarized in Table 12) provide a multidimensional view of gelatin and HPMC capsule behavior under humid conditions. These findings contribute to more informed capsule selection for DPI formulations and highlight the broader need to consider environmental sensitivity in the design and development of drug delivery systems.

Table 12. Performance comparison between gelatin and HPMC capsules under moisture exposure

Characteristic	Gelatin capsules	HPMC capsules
Initial water content	Higher (10-14%)	Lower (4-6%)
Moisture uptake behavior	Steady, gradual absorption	Rapid uptake with fluctuations

Characteristic	Gelatin capsules	HPMC capsules
Moisture at equilibrium	Higher	Lower
Change in hardness	Pronounced softening, occurs early	More gradual and moderate softening
Puncture resistance	High initially, drops	Lower at start, relatively stable
Hydrophilicity	Moderate, decreases with humidity exposure	Strong and consistent
Free volume dynamics	Increase slowly with exposure	Shows greater and more dynamic changes
Mechanical recovery	Partial recovery, slow progression	Partial recovery, but occurs more quickly

To sum up, HPMC capsules showed superior resistance to humidity. While they absorb moisture more quickly, they adapt dynamically at the molecular level, maintaining better mechanical strength and stable surface characteristics. In contrast, gelatin capsules, although initially stronger, experience significant softening and a notable reduction in puncture resistance following moisture exposure, with limited ability to recover their original structure. These distinctions highlight the advantage of HPMC capsules for DPI formulations designed for humid conditions.

6. 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.

7. Summary

DPIs increasingly rely on hard capsules to deliver micronized or engineered particles directly to the lungs, thereby avoiding propellants. While gelatin has been the traditional capsule material for decades, plant-derived capsules have gained interest due to lower residual water content and improved chemical compatibility. However, systematic comparisons of their performance under real-world humidity conditions remain limited. This research investigated the behavior of these two capsules under controlled humidity exposure (25 °C, 75% RH) over durations from 30 minutes to 24 hours, with the goal of identifying the more suitable material for DPI applications. The study aimed to understand how moisture exposure affects the properties of DPI capsules over time, particularly in terms of their capacity to protect moisture-sensitive drug formulations. To assess these effects, moisture uptake was determined gravimetrically by comparing capsule mass before and after storage. Visual assessments of silica beads provided qualitative insights into the degree of moisture absorption. HPMC capsules consistently showed stable moisture uptake and less pronounced color change than gelatin, confirming superior barrier properties. The mechanical properties of the capsules were analyzed using hardness measurements and texture analysis (horizontal deformation, vertical deformation, and puncture testing). Gelatin capsules exhibited significant softening and reduced resistance to deformation with increasing exposure time. In contrast, HPMC capsules maintained their mechanical integrity over time. Surface wettability revealed that gelatin capsules became more hydrophobic after humidity exposure, while HPMC exhibited only minor changes. To explore structural changes at supramolecular level, PALS was used and showed marked alterations in the free volume of gelatin capsules, indicating disruption in the polymer matrix, whereas HPMC capsules remained relatively stable.

Overall, the study showed that gelatin capsules are more sensitive to humidity, exhibiting higher moisture uptake and loss of structural and mechanical integrity. In contrast, HPMC capsules maintained stability under stress, making them more suitable for DPI use in high-humidity environments. By comparing gelatin and HPMC moisture responses, this work aims to support informed DPI capsule selection, helping guide the development of more stable and reliable inhalation products.

8. References

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9. Bibliography of the candidate's publications

9.1. Related to the thesis

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9.2. Other Publications

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